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Isogenic Kidney Glomerulus Chip Engineered from Human Induced Pluripotent Stem Cells

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Abstract

Chronic kidney disease (CKD) affects 15% of the U.S. adult population, but the establishment of targeted therapies has been limited by the lack of functional models that can accurately predict human biological responses and nephrotoxicity. Advancements in kidney precision medicine could help overcome these limitations. However, previously established *in vitro* models of the human kidney glomerulus-the primary site for blood filtration and a key target of many diseases and drug toxicities-typically employ heterogeneous cell populations with limited functional characteristics and unmatched genetic backgrounds. These characteristics significantly limit their application for patient-specific disease modeling and therapeutic discovery.

This paper presents a protocol that integrates human induced pluripotent stem (iPS) cell-derived glomerular epithelium (podocytes) and vascular endothelium from a single patient to engineer an isogenic and vascularized microfluidic kidney glomerulus chip. The resulting glomerulus chip is comprised of stem cell-derived endothelial and epithelial cell layers that express lineage-specific markers, produce basement membrane proteins, and form a tissue-tissue interface resembling the kidney's glomerular filtration barrier. The engineered glomerulus chip selectively filters molecules and recapitulates drug-induced kidney injury. The ability to reconstitute the structure and function of the kidney glomerulus using isogenic cell types creates the opportunity to model kidney disease with patient specificity and advance the utility of organs-on-chips for kidney precision medicine and related applications.

Introduction

Organ-on-a-chip devices are dynamic 3D *in vitro* models that employ molecular and mechanical stimulation, as well as vascularization, to form tissue-tissue interfaces that model the structure and function of specific organs. Previously established organ-on-a-chip devices that aimed to recapitulate the kidney's glomerulus (glomerulus chips)

consisted of animal cell lines[1] or human primary and immortalized cell lines of heterogeneous sources[2,3]. The use of genetically heterogeneous cell sources present variations that significantly limit the studies of patient-specific responses and genetics or mechanisms of disease[4,5]. Addressing this challenge hinges on the availability of isogenic cell lines originating from specific individuals with preserved molecular and genetic profiles to provide a more accurate microenvironment for engineering *in vitro* models[2,3,6]. Isogenic cell lines of human origin can now be easily generated due to advancements in human iPS cell culture. Because human iPS cells are typically noninvasively sourced, can self-renew indefinitely, and differentiate into almost any cell type, they serve as an attractive source of cells for the establishment of *in vitro* models, such as the glomerulus chip[7,8]. The glomerular filtration barrier is the primary site for blood filtration. Blood is first filtered through vascular endothelium, the glomerular basement membrane, and finally through specialized epithelium named podocytes. All three components of the filtration barrier contribute to the selective filtration of molecules. Presented here is a protocol to establish an organ-on-a-chip device interfaced with vascular endothelium and glomerular epithelium from a single human iPS cell source. While this protocol is especially useful to engineer an isogenic and vascularized chip to recapitulate the glomerular filtration barrier, it also provides a blueprint for developing other types of personalized organs-on-chips and multi-organ platforms such as an isogenic 'body-on-a-chip' system.

The protocol described herein begins with divergent differentiation of human iPS cells into two separate lineages - lateral mesoderm and mesoderm cells, which are subsequently differentiated into vascular endothelium and glomerular epithelium, respectively. To generate lateral mesoderm cells, human iPS cells were seeded on basement membrane matrix 1-coated plates and cultured for 3 days (without media exchange) in N2B27 medium supplemented with the Wnt activator, CHIR 99021, and the potent mesoderm inducer, bone-morphogenetic 4 (BMP4). The resulting lateral mesoderm cells were previously characterized by the expression of brachyury (T), mix paired-like homeobox (MIXL), and eomesodermin (EOMES)[9]. Subsequently, the lateral mesoderm cells were cultured for 4 days in a medium supplemented with VEGF165 and Forskolin to induce vascular endothelial cells that were sorted out based on VE-Cadherin and/or PECAM-1 expression using magnetic-activated cell sorting (MACS). The resulting vascular endothelial cells (viEC) were expanded by culturing them on basement membrane matrix 3-coated flasks until ready to seed in the microfluidic device.

To generate mesoderm cells, human iPS cells were seeded on basement membrane matrix 2-coated plates and cultured for 2 days in a medium containing Activin A and CHIR99021. The resulting mesoderm cells were characterized by the expression of HAND1, goosecoid, and brachury (T) as described previously[2,10,11]. To induce intermediate mesoderm (IM) cell differentiation, the mesoderm cells were cultured for 14 days in a medium supplemented with BMP-7 and CHIR99021. The resulting IM cells express Wilm's Tumor 1 (WT1), paired box gene 2 (PAX2), and odd-skipped related protein 1 (OSR-1)[2,10,11].

A two-channel polydimethylsiloxane (PDMS)-based microfluidic chip was designed to recapitulate the structure of the glomerular filtration barrier *in vitro*. The urinary channel is 1,000 µm x 1,000 µm (w x h) and the capillary channel dimension is 1,000 µm x 200 µm (w x h). Cyclic stretching and relaxation cycles were facilitated by the hollow chambers

present on each side of the fluidic channels. Cells were seeded onto a flexible, PDMS membrane (50 µm thick) that separates the urinary and capillary channels. The membrane is outfitted with hexagonal pores (7 µm diameter, 40 µm apart) to help promote intercellular signaling (**Figure 1A**)[2,12]. Two days before IM induction was complete, the microfluidic chips were coated with basement membrane matrix 2. viECs were seeded into the capillary channel of the microfluidic chip using Endothelial Maintenance medium 1 day before IM induction was complete, and the chip was flipped upside down to enable cell adhesion on the basal side of the ECM-coated PDMS membrane. On the day IM induction was completed, the cells were seeded into the urinary channel of the microfluidic chip using a medium supplemented with BMP7, Activin A, CHIR99021, VEGF165, and *all trans* Retinoic Acid to induce podocyte differentiation within the chip. The following day, the media reservoirs were filled with Podocyte Induction medium and Endothelial Maintenance medium, and 10% mechanical strain at 0.4 Hz and fluid flow (60 µL/h) were applied to the chips.

The cellularized microfluidic chips were cultured for 5 additional days using Podocyte Induction medium (in the urinary channel) and Endothelial Maintenance medium (in the vascular channel). The resulting kidney glomerulus chips were cultured for up to 7 additional days in maintenance media for both the podocyte and endothelial cells. The differentiated podocytes positively expressed lineage-specific proteins, including podocin and nephrin[13,14], while viECs positively expressed the lineage identification proteins PECAM-1 and VE-Cadherin, all of which are essential molecules for maintaining the integrity of the glomerular filtration barrier[15,16]. The podocytes and viECs were both found to secrete the most abundant glomerular basement membrane protein, collagen IV, which is also important for tissue maturation and function.

The three-component system of the filtration barrier - endothelium, basement membrane, and epithelium - in the glomerulus chips were found to selectively filter molecules and respond to a chemotherapeutic, nephrotoxic drug treatment. Results from the drug treatment indicated that the glomerulus chip can be used for nephrotoxicity studies and for disease modeling. This protocol provides the general guideline for engineering a functional microfluidic kidney glomerulus chip from isogenic iPS cell derivatives. Downstream analyses of the engineered chip can be carried out as desired by the researcher. For more information on using the glomerulus chip to model drug-induced glomerular injury, refer to previous publications[2,12].

Protocol

1. Prepare basement membrane matrix solutions and coated substrates

1. Thaw basement membrane matrix 1 overnight on ice at 4 °C. Aliquot according to the manufacturer's suggestion for dilution ratio. Using a 50 mL conical tube and pipette, thoroughly mix an appropriate amount of basement membrane matrix 1 into 25 mL of cold DMEM/F12 until completely thawed and dissolved.
    1. To dissolve a frozen aliquot, take ~200 µL from the 25 mL of cold DMEM/F12 and transfer it to the frozen aliquot tube. Pipette up and down until the matrix is thoroughly thawed and dissolved. Transfer the full tube content of basement membrane matrix 1 into the rest of the cold DMEM/F12.

2. Pipette 1 mL of basement membrane matrix 1 solution into each well of a 6-well plate. To use the coated plates the same day, incubate at 37 °C for 2 h.
    1. Alternatively, the coated plates can be wrapped with parafilm and stored at 4 °C for up to 2 weeks. When ready to use the stored plate, incubate at 37 °C for 30 min.
3. Dilute basement membrane matrix 2 in 9 mL of sterile distilled water to achieve a final concentration of 5 µg/mL. Pipette 700 µL of basement membrane matrix 2 solution into each well of a 12-well plate. Wrap the basement membrane matrix 2-coated plates with parafilm and store at 4 °C for up to 2 weeks.
4. Reconstitute lyophilized basement membrane matrix 3 to achieve a final concentration of 1 mg/mL in phosphate-buffered saline (PBS, Ca^{+2}-, and Mg^{+2}-free) as suggested by the manufacturer. Dilute one 250 µL aliquot to a final concentration of 25 µg/mL in 9.75 mL of PBS (Ca^{+2} and Mg^{+2} free). Use 6 mL of this solution to coat one T75 flask (a final concentration of 2 µg/cm^2). Store the matrix-coated flasks at 4 °C for up to 1 week.

2. Human iPS cell culture

NOTE: The DU11 line used in this protocol was tested and found to be free of mycoplasma and karyotype abnormalities.

1. Incubate basement membrane matrix 1-coated plates at 37°C for 1-2 h.
2. Wash each well of the 6-well plate 3x with 1 mL of warm (37 °C) DMEM/F12. Add 2 mL of human iPS cell culture medium (CCM) (**Supplemental Table S1**) to each well of the 6-well plate. Incubate the plates at 37 °C while cells are being prepared for seeding as described below.
3. Wash each well of the 6-well plate containing human iPS cells with 1 mL of warm DMEM/F12.
4. Aspirate the DMEM/F12. Add 1 mL of warm cell detachment buffer to each well of the 6-well plate. Incubate at 37 °C for 1 min.
5. Visually inspect each well under a microscope for dissociation around the cell colony edges. Carefully aspirate the cell detachment buffer from the cells. Gently wash each well with 1 mL of warm DMEM/F12.
6. Inspect the plates under the microscope to ensure that the cells have not completely detached from the plate or been accidentally aspirated.
7. Add 3 mL of warm human iPS CCM to each well of the 6-well plate with cells. Using a cell lifter, gently scrape the colonies. Using a 5 mL serological pipette, gently mix the cell suspension in each well up and down once.
8. Take out the new basement membrane matrix 1-coated plate from the incubator. Transfer 0.5 mL of the cell suspension to each well of the new basement membrane matrix 1-coated plate. Move the plate in a figure-of-eight motion to distribute the cells evenly. Incubate at 37 °C in a 5% CO_2 incubator.
9. Aspirate the spent medium and replace with 3 mL of human iPS CCM every day of culture until cells are 70% confluent (approximately 4 days after passage).

3. Days 0-16: differentiation of human iPSCs into intermediate mesoderm cells

1. Days 0-2: mesoderm induction
    1. Move basement membrane matrix 2-coated plates from 4 °C to room temperature for 2 h to equilibrate after storage.

2. Aspirate the spent medium from each well of the 6-well plate containing approximately 70% confluent human iPS cells. Gently wash the cells 3x with 1 mL of warm DMEM/F12.
3. Aspirate the DMEM/F12. Add 1 mL of cell detachment buffer to each well of the 6-well plate of human iPS cells. Incubate at 37 °C for 5-7 min.
4. Visually inspect each well under a microscope for dissociation around the cell colony edges. Using a cell lifter, gently scrape the colonies.
5. Transfer the cell suspensions from all the wells of the 6-well plate to a 15 mL conical tube and use a P1000 to pipette up and down several times to obtain a single-cell suspension of the iPS cells.
6. Fill the cell suspension in the conical tube to 14 mL with DMEM/F12. Centrifuge for 5 min at 200 × *g* at room temperature.
7. Aspirate the supernatant. Resuspend the cell pellet in 14 mL of warm DMEM/F12. Repeat centrifugation for 5 min at 200 × *g* at room temperature.
8. Aspirate the supernatant. Resuspend the cells in 1 mL of Mesoderm Induction medium (**Supplemental Table S1**). Count the cells using a hemocytometer. Dilute in Mesoderm Induction medium to achieve a final concentration of 1×10^5 cells/mL.
9. Aspirate the coating from the basement membrane matrix 2-coated plate. Rinse each well of the basement membrane matrix 2-coated plate 2x with 1 mL of warm DMEM/F12.
10. Gently pipette the cell suspension up and down 2x. Transfer 1 mL of the cell suspension to each well of the basement membrane matrix 2-coated plate. Gently move the plate in a figure-of-eight motion to distribute the cells evenly.
11. Incubate the plate at 37 °C overnight. The next day (day 1), aspirate the spent medium from each well of the 12-well plate. Replace with 1 mL of warm Mesoderm Induction medium. Incubate at 37 °C overnight.

2. Days 2-16: intermediate mesoderm induction
    1. Aspirate the spent Mesoderm Induction medium. Replace with 1m L of warm Intermediate Mesoderm Induction medium (**Supplemental Table S1**). Aspirate the spent medium and replace with 1 mL of warm Intermediate Mesoderm Induction medium every day for 14 days.

4. Days 0-15: differentiation and expansion of human iPSCs into vascular endothelial cells

1. Day 0: human iPS cell seeding
    1. Prepare 15 mL of human iPS CCM with ROCK Inhibitor (**Supplemental Table S1**). Keep warm at 37 °C.
    2. Incubate one basement membrane matrix 1-coated plate for 1-2 h at 37 °C. Aspirate the basement membrane matrix 1. Wash 3x with 1 mL of warm DMEM/F12.
    3. Aspirate the DMEM/F12. Add 2 mL of human iPS CCM with ROCK Inhibitor to each well of the 6-well plate. Incubate the plates at 37 °C while the cells are being prepared for seeding as described below.
    4. Aspirate the spent medium from each well of the 6-well plate containing approximately 70% confluent human iPS cells. Gently wash the cells 3x with 1 mL of warm DMEM/F12.

5. Aspirate the DMEM/F12. Add 1 mL of cell detachment buffer to each well of the 6-well plate of human iPS cells. Incubate at 37 °C for 5-7 min to dissociate the cells into single cells.
   NOTE: Due to inherent differences between iPS cell lines, the user will need to visually inspect the cells after 5 min to determine the optimal incubation time.
6. Transfer the cells to a 15 mL conical tube. Bring the cell suspension up to 14 mL with warm DMEM/F12 to neutralize the detachment buffer. Centrifuge for 5 min at 200× *g* at room temperature.
7. Gently aspirate the supernatant. Resuspend the cells in 1 mL of Human iPS CCM with ROCK inhibitor. Count the total number of cells using a hemocytometer.
8. Seed the cells between 37,000 to 47,000 cells/cm^2 (355,200 to 451,200 cells/well of a 6-well plate). Incubate at 37 °C overnight.
   NOTE: Due to inherent differences between iPS cell lines, the user will need to determine the optimal seeding density.

2. Days 1-3: lateral mesoderm induction
   1. The next day (day 1), aspirate the spent medium from each well of the 6-well plate of human iPS cells. Replace each well of the 6-well plate with 5 mL of Lateral Mesoderm Induction medium (**Supplemental Table S1**). When scaling up the culture vessel (e.g., flasks), generally, replace with 3x the working volume in the culture vessel.
   2. Do not change this medium for 3 days.
      NOTE: Lateral Mesoderm Induction Medium in the wells will normally change color from red to yellow as the cells use up the nutrients. However, cloudy medium or medium with bacterial growth is not normal and should be decontaminated and discarded as such.
3. Days 4-6: endothelial cell induction
   1. Aspirate the spent medium from each well of the 6-well plate. Replace each well of the 6-well plate with 3 mL of warm Endothelial Induction medium (**Supplemental Table S1**). Incubate the plate at 37 °C overnight.
   2. For the following 2 days (days 5 and 6), collect the spent medium from all wells into a 50 mL conical tube. Store the conical tube at 4 °C. Replenish the cells with 3 mL of warm Endothelial Induction medium. Incubate the plate at 37 °C.
4. Day 7: endothelial cell (viEC) sorting
   1. Take out basement membrane matrix 3 flasks and leave at room temperature for 1 h.
   2. Prepare 50 mL of MACS buffer (**Supplemental Table S1**).
   3. Place cell detachment buffer, MACS buffer, Endothelial CCM, and PBS (Ca^{2+} and Mg^{2+} free) on ice in the tissue culture hood.
   4. Place the magnet on the MACS stand. Place two 50 mL conical tubes and one 15 mL conical tube in a conical tube holder.
   5. Place the MACS stand (with magnet attached) and one LS column in the tissue culture hood. Set up the conical tube holder (with conical tubes inside) on the MACS stand, underneath the magnet (**Supplemental Figure S1A**).
   6. Collect the spent medium from each well of the 6-well plate into the 50 mL conical tube from step 4.3.2.

Wash each well of the 6-well plate with PBS (Ca^{2+} and Mg^{2+} free).

7. Aspirate the PBS. Add 1 mL of cell detachment buffer. Incubate at 37 °C for 5-7 min to fully dissociate the cells.

8. Transfer the cells to a 50 mL conical tube. Bring the cell suspension to 15 mL with cold Endothelial CCM. Centrifuge at 200 × *g* for 5 min at room temperature.

9. Aspirate the supernatant. Resuspend the cells in 1 mL of cold MACS buffer. Count the cells with a hemocytometer.

10. Add 10 mL of MACS buffer to the cell suspension. Centrifuge for 5 min at 200 × *g* at room temperature.

11. Aspirate the supernatant. Resuspend the cells in 80 µL of MACS buffer per 10 million cells. Add 20 µL per 10 million cells of FcR Blocking Reagent, CD31 microbeads, and CD144 microbeads. Incubate for 15 min on ice.

12. While the cell suspension is incubating, centrifuge the medium collected from endothelial induction (step 4.3.2) at 200 × *g* for 10 min.

13. Collect the supernatant in a 500 mL 0.22 µm vacuum filter. Prepare the Endothelial Conditioned medium (**Supplemental Table S1**). Filter the medium under sterile conditions and keep the medium warm at 37 °C.

    NOTE: Endothelial cell proliferation rate will start to decrease after passage 3. To achieve exponential growth after passage 3, endothelial conditioned and maintenance medium may be supplemented with 10 µM TGF-Beta inhibitor (SB431542) starting from passage 1.

14. After the 15 min incubation in step 4.4.11, add 10 mL of MACS buffer per 10 million cells (maximum 30 mL MACS buffer) to the cell suspension. Centrifuge at 200 × *g* for 5 min.

15. Aspirate the supernatant. Resuspend in 1 mL of MACS buffer.

16. Take the LS column and pull the plunger out of the syringe. Place the plunger back into the plastic sleeve. Place the column onto the magnet.

17. Position the first 50 mL conical tube under the column. Add 1 mL of MACS buffer to the column. Collect in the 50 mL conical tube underneath.

18. Let the liquid flow through, although not completely to prevent the column from drying. When the liquid drops begin to trickle slowly, add the cell suspension to the column. Collect the flowthrough in the same 50 mL conical tube.

19. When the liquid drops begin to trickle slowly, position the next 50 mL conical tube under the column. Add the initial flowthrough (step 4.4.18) to the column. Collect in the 50 mL conical tube underneath.

20. When the liquid drops begin to trickle slowly, add 500 µL of MACS buffer 3x to wash the column.

21. Remove the column from the magnet. Place the column on the 15 mL conical tube. Add 1 mL of cold PBS to the column.

22. To collect the cells, take the plunger from the plastic sleeve and firmly push it into the column.

23. Count the cells using a hemocytometer. Bring the cell suspension to 5 mL with PBS. Centrifuge for 5 min at 200 × *g*.

24. While the cells are undergoing centrifugation, wash the basement membrane matrix 3-coated flask 3x with 5 mL of PBS to prepare for cell seeding. Aspirate the PBS and add 20 mL of Endothelial Conditioned medium to the basement membrane matrix 3-coated flask.
25. Remove the cells from the centrifuge and aspirate the supernatant. Resuspend the cells in Endothelial Conditioned medium to be seeded at 26,000 cells/ cm^2 (1.95×10^6 cells/T75 flask). Seed the cells.
26. To calculate the sorting efficiency and cell yield, divide the cell count from step 4.4.23 by the cell count from step 4.4.9.

5. Days 8-15: expansion of viECs
    1. The next day (day 8), aspirate the spent medium from the flask. Replace with 10 mL of Endothelial Conditioned medium. Replace the Endothelial Conditioned medium every other day until the flask is 90% confluent or until the Endothelial Conditioned medium bottle is completely used.
    2. Aspirate the spent medium and replace with 10 mL of Endothelial Maintenance medium (**Supplemental Table S1**) every other day for continued expansion.
    3. To passage the viECs, prepare two basement membrane matrix 3-coated T75 flasks. Leave the flasks at room temperature for 1 h. Thoroughly wash the freshly prepared flasks 3x with 5 mL of PBS.
    4. Aspirate the PBS. Add 10 mL of warm Endothelial Maintenance medium to each freshly prepared flask. Incubate the plates at 37 °C while the cells are being prepared for seeding as described below.
    5. Add 5 mL of cell detachment buffer to a 90% confluent T75 flask of viECs. Incubate at 37 °C for 5-7 min. Transfer the cells to a 15 mL conical tube. Add 5 mL of warm DMEM/F12. Centrifuge at 200 × *g* for 5 min.
    6. Aspirate the supernatant. Resuspend in 1 mL of warm Endothelial Maintenance medium. Add 500 µL of the cell suspension to each of the freshly prepared-T75 flasks.
    7. The next day, aspirate the spent medium. Replace with 10 mL of Endothelial Maintenance medium. Aspirate the spent medium and replace with 10 mL of Endothelial Maintenance medium every other day until the flask is 90% confluent.

5. Day 14: preparation of microfluidic organ chip devices for cell culture

1. Plasma treatment and basement membrane matrix 2 coating
    1. Prepare basement membrane matrix 2 solution (step 1.3). Set it aside.
    2. In a sterile tissue culture hood, unpack the sterile 100 mm x 15 mm round Petri dish. Place the Petri dish top, facing downwards, under the Petri dish bottom (**Supplemental Figure S1B**).
    3. Using tweezers, take out the microfluidic chips from the package and place them inside the Petri dish. Close the Petri dish using the lid from underneath the dish.
    4. At the plasma asher, place the Petri dish lid under the dish, top facing downwards, keeping them together as one unit. Place the Petri dish unit in the

plasma asher chamber. Start the plasma asher with oxygen at 100 W, 15 SCCM, 30 s.

5. **Time sensitive**: Once the treatment is complete, take out the Petri dish unit from the plasma asher. Quickly and lightly wipe the Petri dish lid with 70% ethanol sprayed on a laboratory wipe. Cover the Petri dish with its lid.

6. Bring the Petri dish to the sterile tissue culture hood. Gently add 25 µL of basement membrane matrix 2 solution into the urinary (top) channel of the chip. Add 20 µL of basement membrane matrix 2 solution into the capillary (bottom) channel of the chip.

7. Take two sterile 15 mL conical tube caps and fill them with sterile distilled water (~500 µL). Place the cap in the Petri dish to prevent the chip channels and membrane from drying out. Place the lid on the dish. Incubate at 37 °C overnight.

6. Seeding of viECs and intermediate mesoderm cells into the microfluidic devices

1. Day 15: viECs

    1. Aspirate the medium from T75-flasks containing 90% confluent viECs. Add 5 mL of cell detachment buffer and incubate at 37 °C for 5-7 min.

    2. Transfer the cells to a 15 mL conical tube. Add 5 mL of DMEM/F12. Centrifuge at 200 × *g* for 5 min.
    NOTE: Each T75 flask with approximately 90% confluent viECs will yield ~3 million cells.

    3. Aspirate the supernatant. Resuspend the cells in 300 µL of Endothelial Maintenance medium to obtain approximately 2 million cells/300 µL. Count the cells with a hemocytometer. Set the cell suspension aside.

    4. Transfer the Petri dish containing the microfluidic chips to the tissue culture hood. Attach a P200 barrier tip to the tip of an aspirator.

    5. Flush both the top and bottom channels of the microfluidic chip with 200 µL of DMEM/F12 while simultaneously aspirating the periphery of the outlet.

    6. Hold the chip firmly with the P200 barrier tip attached to the aspirator, away from the outlet of the bottom channel. Firmly inject 20 µL of the viEC suspension with approximately 134,000 cells into the capillary (bottom) channel of the chip. Carefully aspirate medium from the periphery of the outlet.

    7. Check under the microscope for bubbles or an uneven viEC seeding density.

    8. Gently flip the chip over to invert it so that the viECs can adhere to the basal side of the flexible PDMS membrane. Place the chip into the holder cartridge. Add 3 mL of PBS into the chip holder cartridge to prevent the membrane from drying. Incubate the chip at 37 °C for 3 h.

    9. Check the bottom channel under the microscope for a confluent layer of viECs attached to the flexible PDMS membrane. Drop 200 µL of Endothelial Maintenance medium on the inlet of the bottom channel and allow it to flow through the channel to wash the channel of unattached endothelial cells while carefully aspirating from the periphery of the capillary (bottom) channel outlet.

    10. Replace the chip back into the holder cartridge. Incubate at 37 °C overnight.

2. Day 16: intermediate mesoderm (IM) cells

1. Gently flush the capillary (bottom) channel with 200 µL of Endothelial Maintenance medium while carefully aspirating the periphery of the outlet port.
2. Flush the urinary (top) channel with 200 µL of DMEM/F12 while carefully aspirating the periphery of the outlet. Drop ~50 µL of DMEM/F12 on the inlet and outlet ports.
3. Aspirate the Intermediate Mesoderm Induction medium from each well of the 12-well plate.
   NOTE: Each well at the end of differentiation gives approximately 1.5 million IM cells.
4. Add 1 mL of Trypsin-EDTA to each well of the 12-well plate and incubate at 37 °C for 5 min.
5. Gently scrape the cells using a cell lifter, and pipette up and down to dissociate the cells using a P1000. Add 2 mL of Trypsin Neutralization solution (**Supplemental Table S1**) to each well. Transfer the cells to a 50 mL conical tube. Bring the cell suspension volume to 50 mL with DMEM/F12 and centrifuge at 200 × *g* for 5 min.
6. Aspirate the supernatant. Resuspend the cells in 500 µL of Intermediate Mesoderm Induction medium to obtain approximately 3 million cells/500 µL. Count the cells with a hemocytometer.
7. Hold the chip firmly with the P200 barrier tip attached to the aspirator, away from the outlet of the urinary (top) channel. Firmly inject 25 µL of cell suspension with approximately 112,500 IM cells into the urinary (top) channel of the chip, and carefully aspirate the medium from the periphery of the outlet.
8. Check under the microscope for bubbles or an uneven IM cell seeding density. Add 3 mL of PBS into the chip holder cartridge. Incubate at 37 °C for 3 h.
9. Flush both channels with 200 µL of their respective cell culture medium while carefully aspirating the periphery of the chip outlets to help prevent backward flow of the spent medium and cell debris.
10. Attach empty P200 barrier tips into both outlets of the urinary and capillary channels. Pipette 200 µL of Endothelial Maintenance medium and inject half of it into the capillary channel inlet. Release the pipette tip inside the inlet such that both the inlet and outlet of the channel are now attached to pipette tips filled with medium.
11. Pipette 200 µL of IM maintenance medium and inject half into the urinary channel inlet. Release the pipette tip inside the inlet such that both the inlet and outlet of the channel are now attached to pipette tips filled with medium. Incubate the chips with tips embedded at 37 °C overnight.

3. Connect chips to Organ Chip Bioreactor to apply fluid flow and mechanical strain.
   1. Remove P200 tips from the urinary and capillary channels. Add droplets of respective media to the inlet and outlet of the urinary and capillary channels to prevent drying.
   2. Add 3 mL of warm Podocyte Induction medium (**Supplemental Table S1**) to the urinary inlet reservoir. Add 3 mL of warm Endothelial Maintenance medium to the capillary inlet reservoir.
   3. Add 300 µL of warm Podocyte Induction medium to the urinary channel outlet reservoir directly over the outlet port. Add 300 µL of warm Endothelial

Maintenance medium to the capillary channel outlet reservoir directly over the outlet port.

4. Slide the pods onto the tray and into the Organ Chip Bioreactor.
5. Use the **rotary** dial on the Organ Chip Bioreactor to select and start the Prime cycle (2 min). Visually inspect the underside of the pod for small droplets at all four fluidic ports.
6. To achieve fluid-fluid contact between the Pod underside and microfluidic chip ports, gently slide the chip carrier into the Pod. Gently press the chip carrier tab in and up. Aspirate excess medium from the chip surface.
7. Set the Organ Chip Bioreactor flow rate to **60 µL/h**. Set the cyclic strain to **10% at 0.4 Hz**. Use the rotary dial on the Organ Chip Bioreactor to select the **Regulate cycle** and run for **2 h**.
8. Visually inspect the outlet reservoirs for an increase in the level of medium.
9. Use the **rotary dial** on the Organ Chip Bioreactor to select the **Regulate cycle**.

7. Days 17-21 and beyond: podocyte induction and chip maintenance

1. Aspirate the medium from the urinary channel outlet reservoirs diagonally away from the port but keep some medium in the reservoir every day of culture. Replenish the urinary channel inlet reservoir with up to 3 mL of Podocyte Induction medium every 2 days for 5 days.
    1. After 5 days, aspirate the medium from the urinary channel but keep some medium in the reservoir. Replenish the urinary channel inlet reservoir daily with 3 mL of Podocyte Maintenance medium.
2. Aspirate the medium from the capillary channel outlet reservoirs diagonally away from the port but keep some medium in the reservoir every day of culture. Replenish the capillary channel inlet reservoir daily with up to 3 mL of Endothelial Maintenance medium.

8. Functional assay and immunofluorescence imaging

NOTE: See **Supplemental File 1** for details about flow cytometry analysis, ELISA for chip effluent, and mRNA isolation.

1. Functional assay (molecular filtration) using inulin and albumin
    1. Aspirate the medium from the capillary channel outlet reservoir diagonally away from the port but keep some medium in the reservoir. Replace with 3 mL of Endothelial Maintenance medium supplemented with inulin and albumin (**Supplemental Table S1**) for 6 h.
    2. Using a 5 mL serological pipette, measure the volume (in mL) of medium from the urinary channel and transfer to a 15 mL conical tube. Wrap the tube in aluminum foil to protect from light and minimize photobleaching of the fluorophore-conjugated inulin and albumin. Replenish the reservoir with 3 mL of Podocyte Maintenance medium.
    3. Prepare a stock solution of inulin in Podocyte Maintenance medium. Starting from 25 µg/mL Inulin, prepare eight standards of inulin *via* a 2x serial dilution in Podocyte Maintenance medium.
    4. Similarly, prepare a stock solution of albumin in Podocyte Maintenance medium. Starting from 150 µg/mL albumin, prepare eight standards of albumin

via a 2x serial dilution in Podocyte Maintenance medium.

5. Pipette in duplicate 100 µL of each standard inulin concentration into each well of a black, 96-well plate (or 16 total wells of inulin). Pipette in duplicate 100 µL of each standard albumin concentration into each well of the same 96-well plate (16 total wells of albumin). Pipette in duplicate 100 µL Podocyte Maintenance medium to serve as the blank (or two total wells of Podocyte Maintenance medium).
6. Pipette in duplicate 100 µL of effluent medium from the urinary channel into each well of the same 96-well plate. Pipette in duplicate 100 µL of effluent medium from the capillary channel.
7. Insert the plate into the plate reader and measure the fluorescence for albumin at excitation 550 nm and emission 615 nm. Measure the same plate for inulin at excitation 513 nm and emission 577 nm.
8. From the generated data, average the duplicate measurements (per chip). Subtract the blank value corresponding to that plate reading from the rest of the data on the sheet.
9. Plot the values corresponding to the albumin standards to create a standard curve with concentration [µg/mL] on the x-axis and fluorescence on the y-axis. Plot the values corresponding to the inulin standards to create a standard curve with concentration [µg/mL] on the x-axis and fluorescence on the y-axis.
10. Use a statistical analysis software package and linear interpolation to determine the urinary concentration of inulin and albumin, respectively, in the effluent medium from the chips.
11. Determine the urinary clearance of inulin/albumin from the chips using equation (**1**)[2]:

    Urinary Clearance = ([U] × UV) / [P] (**1**)

    Where [U] is the urinary concentration from step 8.1.10, UV is the volume of collected urinary channel effluent from step 8.1.2, and [P] is the capillary concentration of inulin or albumin (10 µg/mL inulin or 100 µg/mL albumin).

2. Immunofluorescence imaging
    1. Inject an empty P200 tip into the outlet ports of the urinary and capillary channels. To fix the cells, pipette 200 µL of 4% formaldehyde and inject half of it into the bottom channel inlet. Release the pipette tip inside the inlet.
    2. Pipette 200 µL of 4% formaldehyde and inject half of it into the urinary channel inlet. Release the pipette tip inside the inlet such that both the inlet and outlet of the channel are now attached to pipette tips filled with fixative.
    3. Incubate the chips at room temperature for 20 min.
    4. After 20 min, discard all the pipette tips. Inject clean, empty P200 tips into the outlet ports of the urinary and capillary channels. To permeabilize the cells, pipette 200 µL of 0.125% Triton X-100/PBS and inject half of it into the capillary channel inlet. Release the pipette tip inside the inlet.
    5. Pipette 200 µL of 0.125% Triton X-100/PBS and inject half of it into the urinary channel inlet. Release the pipette tip inside the inlet. Incubate the chip at room temperature for 5 min.
    6. Discard all the pipette tips. Inject clean, empty P200 tips into the outlet ports of the urinary and capillary

channels. To block the cells, pipette 200 µL of 1% bovine serum albumin (BSA) in 0.125% Triton X-100/PBS and inject half of it into the capillary channel inlet. Release the pipette tip inside the inlet.

7. Pipette 200 µL of 1% BSA in 0.125% Triton X-100/PBS and inject half of it into the urinary channel inlet. Release the pipette tip inside the inlet. Incubate at room temperature for 30 min.

8. Discard all the pipette tips. Wash both channels 3x by pipetting 200 µL of 0.125% Triton X-100/PBS into each channel and incubating at room temperature for 5 min.

9. Prepare 100 µL of primary antibody per channel with the dilution recommended by the manufacturer in 0.125% Triton X-100/PBS. Inject clean, empty P200 tips into the outlet ports of the urinary and capillary channels. Pipette 100 µL of primary antibody and inject half into the respective channels. Release the pipette tip inside the inlet.

10. Incubate for 1 h at room temperature or, for better results, overnight at 4 °C.
    NOTE: Multiple primary antibodies can be applied at once; however, the primary antibody solution must be diluted according to the manufacturer's instructions.

11. Wash the channels 3 x 10 min as described in step 8.2.8.

12. Prepare 100 µL of secondary antibody per channel with the dilution factor recommended by the manufacturer in 0.125% Triton X-100/PBS. Inject clean, empty P200 tips into the outlet ports of the urinary and capillary channels. Pipette 100 µL of secondary antibody and inject half into the respective channels. Release the pipette tips inside the inlet. Incubate for 1 h at room temperature.
    NOTE: Each secondary antibody must be applied separately. Wash at least 3 x 10 min between each application according to step 8.2.8.

13. Wash the channels 3 x 10 min as described in step 8.2.8.

14. Flush both channels once with 200 µL of distilled water while aspirating the residual fluid from the periphery of the chip outlet ports.

15. Inject clean, empty P200 into the outlet ports of the urinary and capillary channels. To counterstain the cells, pipette 200 µL of 4',6-diamidino-2-phenylindole (DAPI, 1:1,000 dilution in distilled water) and inject half of it into the capillary channel inlet. Release the pipette tip inside the inlet, and pipette 200 µL of DAPI and inject half of it into the urinary channel inlet. Release the pipette tip inside the inlet, and incubate at room temperature for 5 min.

16. Discard all the pipette tips. Inject clean, empty P200 tips into the outlet ports of the urinary and capillary channels. To counterstain the cells, pipette 200 µL of phalloidin (1:1,000 dilution in PBS without Ca^{2+} and Mg^{2+}) and inject half of it into the capillary channel inlet and release the pipette tip inside the inlet. Pipette 200 µL of phalloidin (1:1,000 dilution in PBS without Ca^{2+} and Mg^{2+}) and inject half of it into the urinary channel inlet and release the pipette tip inside the inlet. Incubate at room temperature for 15 min.

17. Flush the channels 3x with 200 µL of PBS without Ca^{2+} and Mg^{2+}, and aspirate excess fluid from the periphery of the outlet ports.

18. Visualize the chips.

Representative Results

Here we show that a functional 3D *in vitro* model of the glomerulus can be vascularized and epithelialized from an isogenic source of human iPS cells. Specifically, this protocol provides instructions on how to apply human iPS cell technology, particularly their ability to differentiate into specialized cell types, to generate kidney glomerular epithelium (podocytes) and vascular endothelium (viECs) that can be integrated with microfluidic devices to model the structure and function of the human kidney at the patient-specific level. A schematic overview of this protocol and timeline (**Figure 1A**) describes how to culture mitotically active human iPS cells (**Figure 1B**) and then differentiate them (in parallel) into mesoderm and lateral mesoderm cell lineages (**Figure 1C,D**). The resulting mesoderm cells were found to express brachyury (T), while the lateral mesoderm cells expressed brachyury (T), MIXL, and EOMES[2,9,10,11].

Subsequent differentiation of the mesoderm cells produced intermediate mesoderm (IM) cells, while differentiation of the lateral mesoderm cells produced viECs (**Figure 1D**)[2,10,11,17]. Flow cytometry analysis was used to examine the expression of CD144 in differentiated viECs (before and after MAC sorting) compared to negative controls (including stained and unstained undifferentiated human iPS cells and unstained endothelium). An optimized endothelial differentiation will result in 50% or greater CD31/CD144-positive cells before MAC sorting, which will significantly improve after cell sorting compared to controls. Representative results show 59% differentiation efficiency for CD144 before MAC sorting, which increased to 77% or more CD144-positive cells (not including CD31-positive cells) after MAC sorting (**Figure 1E**).

On day 14 of this protocol (before the completion of IM differentiation and viEC expansion), the organ-on-a-chip devices were prepared for cell seeding by plasma-treatment and functionalization with basement membrane matrix 2. The following day (day 15 of the protocol), viECs were seeded into the capillary (bottom) channel of the microfluidic device with viEC medium. The day after viEC seeding (day 16 of the protocol), IM cells were seeded into the urinary (top) channel of the microfluidic device with podocyte induction medium. The day following IM cell seeding (day 17 of this protocol), 60 µL/h fluid flow rate and 10% strain at 0.4 Hz were applied to the glomerulus chips. These chips experience shear stress of 0.017dyn cm^{-2} and 0.0007dyn cm^{-2} in the capillary and urinary channels, respectively[2,12]. After up to 5 days of podocyte induction and 6 days of vascular endothelium propagation in the chip (day 21 of this protocol) (**Figure 2A**), the resulting cells within the glomerulus chips expressed lineage identification markers.

Specifically, the podocytes in the urinary channel expressed podocin and nephrin (**Figure 2B**, top panel), and the viECs in the capillary channel expressed PECAM-1 (CD31) and VE-Cadherin (CD144) (**Figure 2B**, bottom panel). Additionally, both the podocyte and viEC layers expressed collagen IV, the most abundant GBM protein (**Figure 2B**) in the kidney glomerulus. More collagen IV is expressed in the urinary channel because podocytes are the predominant producers of collagen IV, including the α3α4α5 isoform, which is the main heterotrimer isoform of collagen in the mature glomerulus. In addition, the podocytes propagated in the glomerulus chips developed foot processes and secreted VEGF165, both of which are characteristic features of

functional models of the kidney glomerulus[2,12]. This protocol also provides an assessment of the selective molecular filtration function of the kidney glomerulus using inulin and albumin, from which the glomerulus chips selectively filter small molecules (inulin) from the capillary into the urinary channel, while preventing large proteins (albumin) from leaving the capillary channel (**Figure 2C**)[2,10,12].

As each human iPS cell line exhibits inherent differences in the doubling time, it is important to note that optimal cell seeding densities for different cell lines may vary and must therefore be optimized by the researcher. For endothelial cell differentiation, if the seeding density of human iPS cells is too low, the researcher may observe a lower yield of differentiated endothelial cells (<30% efficiency). If the seeding density of human iPS cells is too high, the researcher may observe rapid cell overgrowth, detachment or poorer adhesion, increased cell death, and low yield (<30% efficiency). During endothelial induction (days 4-7 of differentiation), an increase in the cell number resulting in a secondary layer of cells is normal but should be kept to a minimum (**Figure 3A**). For IM and podocyte differentiation, overseeding of human iPS cells (>100,000 cells/well of a 12-well plate) may result in IM cells that grow in large clusters or form aggregates, which can impede differentiation and result in podocytes with a less mature morphological phenotype of aggregated cells and less secondary and/or tertiary foot processes[10,11].

During microfluidic chip culture, unexpected fluid crossflow between the urinary and capillary channels (**Figure 3B**) may be observed if there is ruptured or inadequate bonding of the PDMS chip components, or if the path of fluid flow is blocked. This undesired fluid crossflow may also result from a compromised filtration barrier such as tissue models from inadequate (low) cell seeding or damaged cell layers. To prevent this problem, it is recommended that the researcher follows the recommended protocol and cell seeding densities, as well as visually inspects the chips for air bubbles in the channels at every stage of the process. If air bubbles are observed in the media reservoirs of the microfluidic chips that are under fluid flow, the pump can be stopped and the medium degassed under sterile conditions.

Together, this protocol and representative results describe the derivation of vascular endothelium (viECs) and glomerular epithelium (podocytes) from an isogenic human iPS cell line, and their reconstitution in a microfluidic organ-on-a-chip device to recapitulate the structure and function of the kidney glomerular filtration barrier in a patient-specific manner.

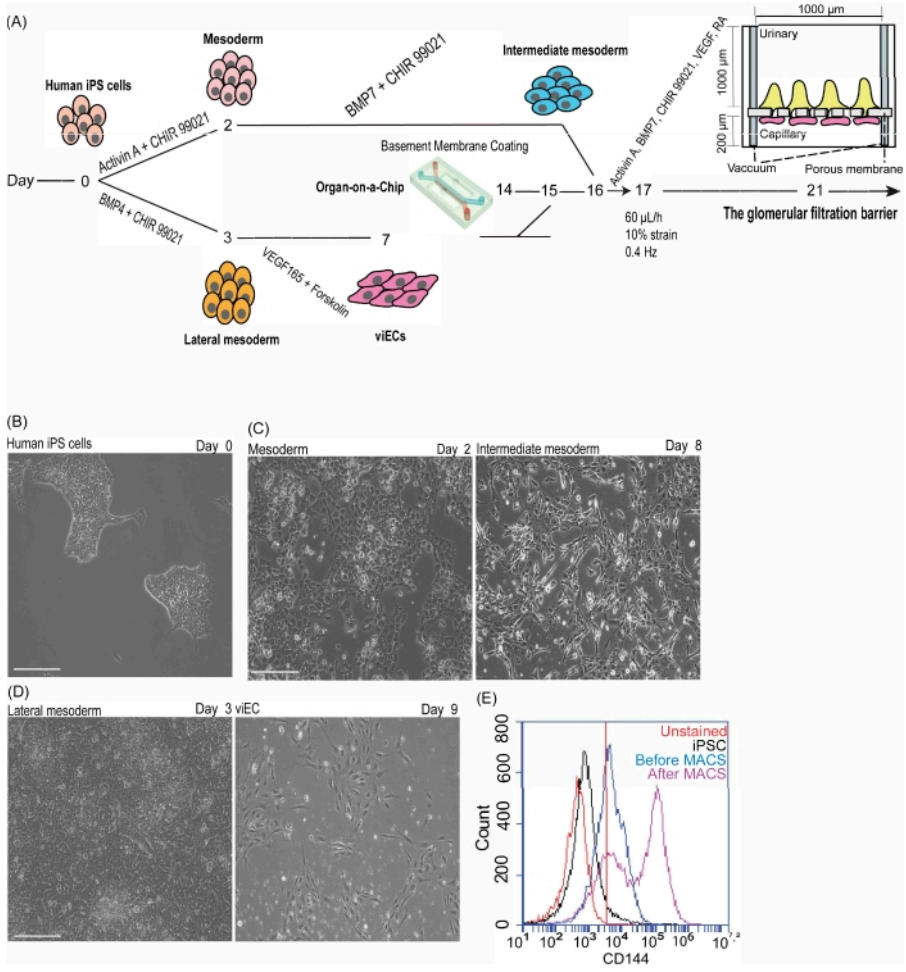

Figure 1: Derivation of isogenic glomerular epithelium and vascular endothelium from human iPS cells. (**A**) Schematic timeline of intermediate mesoderm and viEC induction, organ-on-a-chip design and basement membrane matrix coating, cell seeding into the chip, and podocyte induction within the chip. (**B**) Representative brightfield images of PGP1 human iPS cells before dissociation at day 0 of the protocol. (**C**) Representative brightfield images of PGP1 mesoderm cells on day 2 of differentiation (left) and intermediate mesoderm cells on day 8 of differentiation (right). (**D**) Representative brightfield images of PGP1 lateral mesoderm cells on day 3 of differentiation (left) and PGP1 viECs on day 9 of differentiation (2 days of expansion) (right). Scale bars = 275 µm (**B**-**D**). (**E**) Quantification of viEC differentiation *via* flow cytometry analysis for CD144-positive cells on day 7 of endothelial cell differentiation before MACS (blue) and on day 9 of endothelial cell expansion after MACS (pink) compared to CD144-stained human iPSCs (black) and unstained endothelial cells (red). This figure has been modified from[12]. Abbreviations: iPSCs = induced pluripotent stem cells; viEC = vascular endothelial cells; BMP4/7 = bone morphogenetic protein 4/7; RA = retinoic acid; VEGF = vascular endothelial growth factor; MACS = magnetic-activated cell sorting. Please click here to view a larger version of this figure.

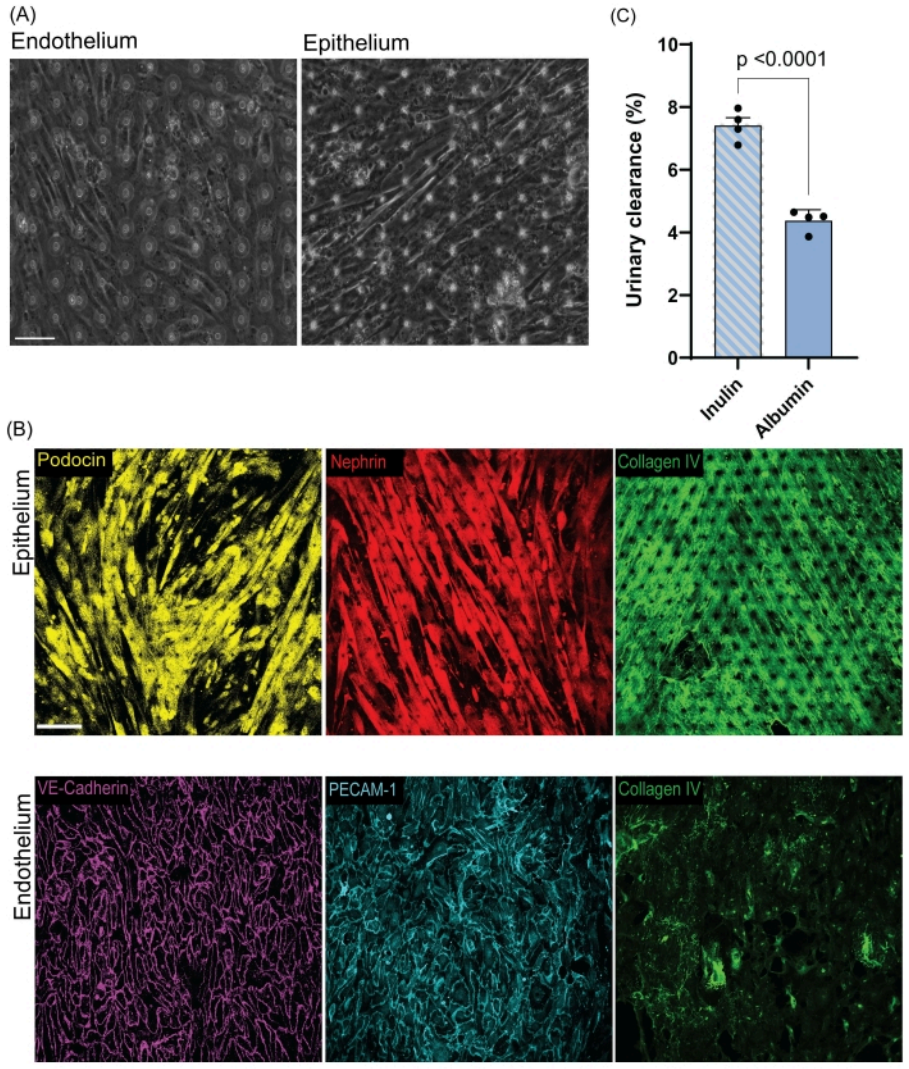

Figure 2: Representative images of vascular endothelium and glomerular epithelium (podocyte layer) cultured within the microfluidic kidney glomerulus chip. Representative brightfield images of viECs (left) and glomerular epithelium (podocytes) (right) propagated in the glomerulus chip. Scale bars = 183 µm. (**B**) Representative immunofluorescent images of glomerular epithelium (podocytes) and viEC showing the expression of lineage-specific markers. Scale bars = 100 µm. (**C**) Representative data showing selective molecular filtration in the glomerulus chip. Error bars represent SD. $p < 0.0001$. This figure has been reproduced from [12]. Abbreviations: viECs = vascular endothelial cells; VE-cadherin = CD144; PECAM-1 (= CD31) = platelet endothelial cell adhesion molecule. Please click here to view a larger version of this figure.

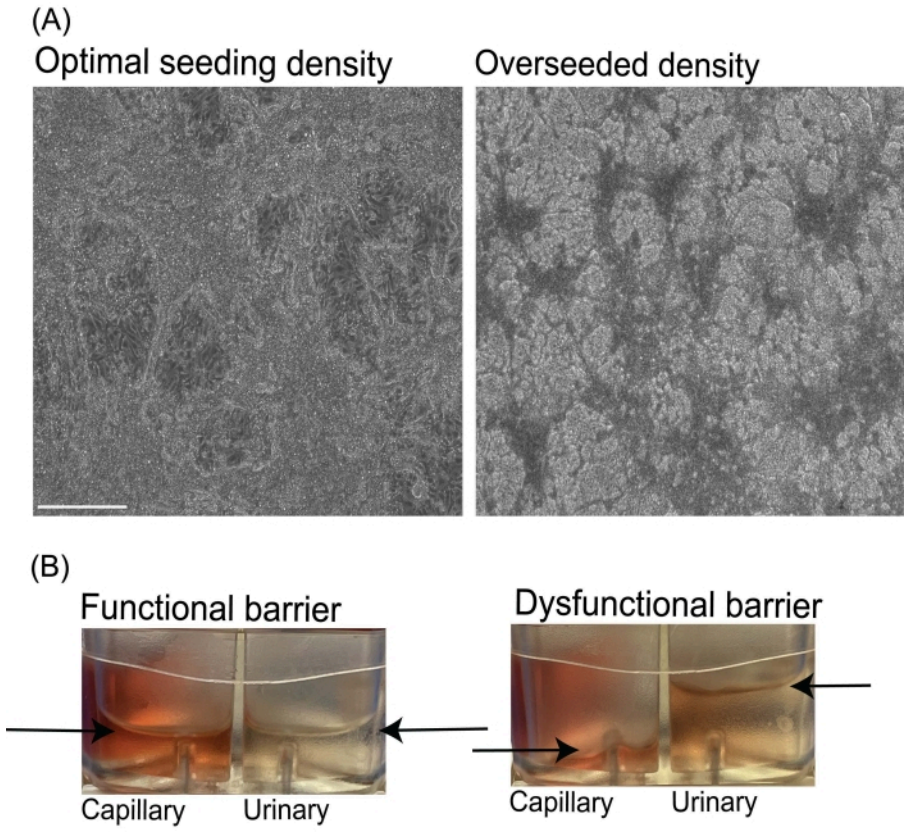

Figure 3: Images of suboptimal endothelium seeding density and uneven fluid flow in the microfluidic chips. (**A**) Representative brightfield images of optimal (left) and overseeded (right) cell cultures on day 6 of viEC differentiation. Scale bars = 275 µm. (**B**) Representative images of outlet reservoirs from microfluidic chips with an even fluid flow and a functional barrier (left). Image from a chip with uneven fluid flow or dysfunctional barrier (right). Arrows denote fluid levels in outlet reservoirs for the capillary and urinary channels of the chips. Abbreviation: viECs = vascular endothelial cells. Please click here to view a larger version of this figure.

Supplemental File 1: Flow cytometry, ELISA for chip effluent, and mRNA isolation. Please click here to download this File.

Supplemental Figure S1: In-tissue culture hood material setups. (**A**) In-tissue culture hood material set up for MACS, including ice bucket with media, magnet on magnet stand, and conical tubes underneath the magnet. (**B**) In-tissue culture hood material setup of Petri dish for chips, with the top, facing downwards, under the Petri dish bottom. Abbreviation: MACS = magnetic-activated cell sorting. Please click here to download this File.

Supplemental Table S1: Media and buffers used in this protocol. Please click here to download this Table.

Discussion

In this report, we outline a protocol to derive vascular endothelium and glomerular epithelium (podocytes) from an isogenic human iPS cell line and the use of these cells to engineer a 3D organ-on-a-chip system that mimics the structure, tissue-tissue interface, and molecular filtration function of the kidney glomerulus. This glomerulus chip is outfitted with endothelium and glomerular epithelium that, together, provide a barrier to selectively filter molecules.

Researchers interested in adapting this protocol should make the following considerations: first, optimization may be necessary for cell seeding depending on the inherent growth characteristics of the stem cell lines being used. Cell seeding density may vary due to intrinsic differences in human iPS cell proliferation rates. It is recommended that researchers begin with the mesoderm seeding density suggested by the protocol, and then adjust if necessary. Similarly, it is recommended that the lateral mesoderm differentiation starts with the suggested cell seeding density before adjusting as needed to achieve a viEC yield and sorting efficiency of 50% or more. If sufficient cells are not differentiated after sorting, TGF-Beta inhibitor (SB431542) can be used to prevent quiescence and help exponentially expand viECs (past passage 3). However, several cellular processes or signaling pathways are dependent on TGF-Beta (e.g., pathogenesis of hyperglycemia/diabetes, immune homeostasis); as such, it is recommended that researchers consider the effects of TGF-Beta inhibition on downstream analysis or ensure adequate testing to avoid unintended experimental outcomes.

Second, it is important to note possible variations in reagent quality and manufacturer specifications, particularly for components acquired from vendors other than those specified by the protocol. Thus, it is recommended that the researcher test reagents from different lot numbers, suppliers, and vendors to ensure reproducibility of experiments and results. Generally, the researcher should avoid excessive exposure of the human iPS cells to dissociation enzymes in the detachment buffers as this can lead to decreased cell viability and altered molecular profile of the cells. Additionally, the podocyte induction medium must be protected from light to prevent inactivation of *all trans* retinoic acid. Third, during organ-on-a-chip cell culture, it is critical to avoid air bubbles in the channels of the microfluidic device when perfusing the chips. The appearance of air bubbles can be minimized or prevented by regularly inspecting the chips during cell seeding, by maintaining liquid-liquid contact at every step involving chip perfusion, and by not pushing or pulling air into the fluidic channels when using pipette tips and/or aspiration.

Previous efforts to engineer kidney glomerulus chips with genetically matched epithelium and endothelium have relied on the use of animal-derived cells[1]. While these animal-derived cell lines have traditionally been used for preclinical studies, they often fail to recapitulate human physiological responses, which contribute to the high failure rate (89.5%) of in-human clinical trials[18]. To help overcome some of these problems, functional *in vitro* models that more closely recapitulate human biology are desirable. Progress has been made to develop multicellular models of the human kidney; however, the glomerulus chips employed human cells from heterogeneous, non-isogenic sources. For example, we previously established a glomerulus chip reconstituted from human iPS cell-derived podocytes and primary tissue-derived endothelium[10]. Studies from other research groups employed a mixture of primary cells[4,9], immortalized cells[3], or amniotic fluid-derived cells[3,6] that

limit their use for studying patient-specific responses or applications in personalized medicine.

The protocol described herein overcomes these limitations by enabling the derivation of both vascular endothelium (viECs) and glomerular epithelium (podocytes) from the same human iPS cell line and integrating these cells into compartmentalized microfluidic organ-on-a-chip devices to model the structure and function of the kidney glomerular capillary wall *in vitro*. Given the unlimited self-renewal of human iPS cells, combined with their ability to differentiate into almost any cell type, this protocol also provides an avenue for continuous sourcing of human podocytes and viECs for tissue engineering and other biomedical applications. This approach for the derivations of podocytes and viECs have been reproduced in multiple patient-specific human iPS cell lines, including PGP1- and DU11[2,10,12,17,19], thus enabling the establishment of personalized kidney glomerulus chips from the desired patient populations.

The strategy for differentiating podocytes within the microfluidic chips enables mechanistic study of the developing human kidney glomerulus and disease modeling. However, the study of the developing human kidney glomerulus is limited by the viECs requiring sorting to enrich for the desired population. This work could benefit from the establishment of methods for differentiation of viECs without a need for subpopulation selection. This study is also limited by the thick PDMS membrane that separates the vascular endothelium and podocyte cell layers. Future work could integrate novel biomaterials to replace the thick PDMS to better mimic the molecular and biophysical properties of the glomerular basement membrane. For example, an alternative membrane could be engineered to possess biodegradable qualities with tunable porosity and be thinner (more GBM-like) than the 50 µm thick PDMS membrane used in this protocol.

Nevertheless, the glomerulus chip produced by this protocol can be applied to study the mechanisms of debilitating kidney diseases and serve as a platform for nephrotoxicity testing and drug discovery. Because human iPS cells maintain the genetic profile of the donor and the glomerulus chip is able to model kidney disease[12], novel therapeutic targets could be discovered in the future to benefit those suffering from hereditary forms of kidney disease. Additionally, patient-specific biological responses to post-transplantation drugs can be more accurately evaluated using an isogenic kidney chip such as the one described in this study. Finally, this glomerulus chip is poised for studying the effects of fluid dynamics and differential mechanical strain-such as those observed in kidney disease patients with hypertension or cardio-renal syndrome-given the relative ease of modulating the rates of fluid flow, tissue stretching, or mechanical strain. It is conceivable that this protocol could advance the current understanding of human kidney development and disease mechanisms, as well as facilitate the development of personalized therapeutics in the future.

Disclosures

S.M. is an inventor on a patent related to podocyte differentiation from human iPS cells. The other author has nothing to disclose.

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