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Title: Isolation of Primary Human Proximal Tubule Epithelial Cells and Their Use in Creating a Microphysiological Model of the Renal Proximal Tubule

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here:

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 31
Number of Shots: 53

Introduction

1.1. **Brooke Chalker:** Our research focuses on the development and application of a microphysiological system that mimics the human renal proximal tubule, enabling more predictive and physiologically relevant in vitro assessments of drug transport, metabolism, and nephrotoxicity.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.4.1, 4.4.2., 4.4.3.*

What are the current experimental challenges?

1.2. **Brooke Chalker:** The current challenges we face with our experiments is maintaining cell viability and function over extended culture time of six months to study chronic drug exposure.

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

What research gap are you addressing with your protocol?

1.3. **Brooke Chalker:** Our research is addressing the need for an accurate in vitro model to accurately predict human kidney-specific responses to drugs, particularly in the context of drug transport, metabolism, and nephrotoxicity.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 3.*

Ethics Title Card

This research was conducted in compliance with the University of Washington's human tissue handling guidelines

Protocol

NOTE: All the footage that the author has provided are lengthy and they have not indicated any timestamps.
For every shot, show a part from the beginning of the process and then show some part from the end of the process.

2. Isolation of PTECs from the Whole Kidney

Demonstrator: Brooke Chalker

2.1. To begin, place the kidney on a round 15-centimeter culture dish **[1]**. Aspirate and discard the remaining media from the dish **[2]**.

2.1.1. Talent positioning the kidney on the dish.

2.1.2. Talent using a pipette to aspirate leftover media from the culture dish and discarding it.

2.2. Using sterile razor blades, remove the surrounding fat and the renal capsule **[1]**. Gently score the renal capsule to create a slit down the center **[2]**.

2.2.1. Talent trimming away fat and capsule using sterile blades.

2.2.2. Talent scoring the capsule with a slit down the center using gentle pressure.

2.3. Using fingers, pull off the capsule **[1]**. Then, with razor blades, slice off any fat attached to the kidney before discarding both the capsule and fat into another culture dish **[2]**.

2.3.1. Talent peeling off the renal capsule by hand.

2.3.2. Talent cutting off excess fat.

2.4. Separate the cortex from the medulla and discard the medulla **[1]**.

2.4.1. Talent separating the cortex tissue from the medulla and discarding the medulla.

2.5. Using a razor blade, mince the tissue into pieces smaller than 1 cubic centimeter **[1]** until a slurry-like appearance is achieved **[2]**.

2.5.1. Talent mincing the cortex tissue.

2.5.2. A shot of the slurry-like appearance.

2.6. Add a small amount of kidney digestion buffer to the dish **[1]**. Transfer the slurry into

the first set of 50-milliliter tubes containing 35 milliliters of the kidney digestion solution **[2]**. Distribute evenly and ensure the total volume in each tube does not exceed 45 milliliters **[3]**.

2.6.1. Talent pipetting buffer into the dish.

2.6.2. Talent transferring the slurry into tubes containing kidney digestion solution.

2.6.3. A shot of the tubes after even distribution of the slurry in the buffer.

2.7. Place the tubes onto a 37 degrees Celsius orbital shaker and incubate for 30 minutes at the highest speed that keeps the tubes secure **[1]**. Spray the tubes with 70 percent ethanol before returning them to the biosafety cabinet **[2]**.

2.7.1. Talent placing the tubes on the shaker and adjusting speed settings.

2.7.2. Talent spraying tubes with ethanol.

2.8. Invert the tubes to mix **[1]** and allow the larger tissue pieces to settle to the bottom **[2]**. Transfer the supernatant to new 50-milliliter conical tubes containing 10 milliliters of fetal bovine serum or FBS *(F-B-S)* without transferring the intact tissue **[3]**.

2.8.1. Talent inverting the tubes in the biosafety cabinet.

2.8.2. A shot of the larger tissue pieces settled at the bottom.

2.8.3. Talent pipetting out supernatant into a new 50-milliliter conical tube containing serum without transferring the intact tissue.

2.9. Centrifuge the tubes at 200 *g* for 7 minutes **[1]**.

2.9.1. Talent placing the tubes in the centrifuge.

2.10. Now, carefully aspirate out the supernatant from each tube **[1]**. Add 10 milliliters of Proximal tubule epithelial cells or PTEC *(p-teck)* media into each tube and resuspend the pellets **[2]**.

2.10.1. Talent removing supernatant using a pipette.

2.10.2. Talent adding PTEC media and resuspending the cell pellet.

2.11. Strain the resulting cell suspensions through 100-micrometer cell strainers into new 50-milliliter conical tubes **[1]**.

2.11.1. Talent pouring cell suspensions through strainers into new 50-milliliter conical tubes.

2.12. Centrifuge the cell filtrates at 300 *g* for 5 minutes **[1]**.

2.12.1. Talent loading tubes into centrifuge and starting spin.

2.13. Next, wash the pellets with 5 milliliters of DPBS and resuspend them using a P1000 *(P-one-thousand)* tip **[1-TXT]**.

2.13.1. Talent adding DPBS to tubes and using a pipette tip to resuspend cell pellets.
TXT: Centrifuge again at 300 *x g* for 5 min

2.14. After centrifuging the tubes again for 5 minutes, aspirate off the supernatant and repeat this step for two more washes **[1]**.

2.14.1. Talent removing supernatant from the tube in the hood.

2.15. Then, resuspend the cell pellets with 15 milliliters of PTEC media **[1]** and plate cells into sterile T-75 cell culture flasks **[2]**.

2.15.1. Talent resuspending the cell pellets with 15 milliliters of PTEC media.

2.15.2. Talent adding solution to T-75 flasks.

2.16. Label the flasks appropriately **[1]** and place them in a sterile incubator set at 37 degrees Celsius with 5 percent carbon dioxide to allow the primary tubular epithelial cells to grow undisturbed for 48 hours before the first media change **[2]**.

2.16.1. Talent applying labels to flasks.

2.16.2. Talent placing flasks inside incubator and closing the door.

3. Passaging and Cryopreservation of PTECs

3.1. Aspirate media from the flask **[1]**. Add 5 milliliters of pre-warmed 0.05 percent Trypsin-EDTA into each T-25 flask **[2]** and incubate at 37 degrees Celsius for 1 to 2 minutes for trypsin digestion **[3]**.

3.1.1. Talent removing old media from T-25 flask.

3.1.2. Talent adding Trypsin-EDTA.

3.1.3. Talent placing the flasks in an incubator.

3.2. Once cells are detached, neutralize trypsin by adding 5 milliliters of pre-warmed defined trypsin inhibitor solution **[1]**.

3.2.1. Talent pipetting trypsin inhibitor solution into the flask.

3.3. Resuspend the mixture five times to dislodge any remaining attached cells **[1]**. Then, transfer the cell suspension into 15-milliliter conical tubes before centrifuging at 400 *g* for 5 minutes **[2]**.

3.3.1. Talent pipetting up and down for resuspension.

3.3.2. Talent transferring the cell suspension into 15-milliliter conical tubes.

3.4. After centrifugation, aspirate the supernatant from the centrifuged tubes **[1]**.

3.4.1. Talent removing supernatant from the tube.

3.5. Resuspend the cell pellets with 14 milliliters of PTEC media per tube **[1]** and transfer the suspensions into T-75 flasks **[2-TXT]**.

3.5.1. Talent resuspending the pellet with 14 milliliters of PTEC media.

3.5.2. Talent transferring suspension into new T-75 flask. **TXT: Use 1 tube per flask**

3.6. Perform a T-shake to evenly distribute cells across the flask **[1-TXT]**.

3.6.1. Talent shaking flask in a "T" motion. **TXT: Monitor the cells and replace media every 48 h**

3.7. For cryopreserving PTECs, resuspend the cell pellets in 2 milliliters of 10 percent dimethyl sulfoxide and 90 percent low glucose PTEC media per 15 milliliter tube **[1]**. Transfer 1 milliliter into each cryovial **[2]**.

3.7.1. Talent adding dimethyl sulfoxide and PTEC media to the tube and resuspending cell pellets.

3.7.2. Talent pipetting 1 milliliter of the suspension into cryovials.

3.8. Transfer the cryovials to a cell freezing container for temperature-controlled cooling **[1]** and place the container in a minus 80 degrees Celsius freezer for 24 hours **[2-TXT]**.

3.8.1. Talent placing cryovials in freezing container.

3.8.2. Talent placing the container in a freezer. **TXT: After 24 h, move the cryovials into a liquid N_2 tank for long-term storage**

4. Seeding Primary PTECs in an MPS Device

4.1. Place a pre-sterilized 5-milliliter syringe with a 22-gauge needle into a 15-milliliter tube containing 5 milliliters of ethanol **[1]**.

4.1.1. Talent inserting syringe with needle into ethanol-filled 15-milliliter tube.

4.2. Take a 15-milliliter conical tube containing the PTEC cell pellet, add 80 microliters of PTEC media to it, and gently resuspend **[1]**.

4.2.1. Talent adding PTEC media to the 15-milliliter conical tube containing the PTEC cell pellet and gently pipetting to resuspend.

4.3. Transfer the resuspended cell solution to a 1.5-milliliter microtube **[1]**.

4.3.1. Talent pipetting the cell suspension into a microtube.

4.4. Now, place a dish containing the microphysiological system or MPS device into the biosafety cabinet **[1]**. Close Ports number 2, 4, and 5, **[2]** and turn the screw valve horizontally to align with the ports **[3]**.

4.4.1. Talent placing the dish containing MPS device in the biosafety hood.

4.4.2. Talent closing Ports number 2, 4, and 5.

4.4.3. Talent adjusting screw valves horizontally to align with the ports.

4.5. Gently flick the PTEC-containing tube to resuspend the cells **[1]**. After filling the syringe with the resuspended cells, insert the needle into the injection port farthest from the screw valve ports **[2]**.

4.5.1. Talent flicking the PTEC-containing tube to resuspend cells.

4.5.2. Talent inserting the needle of the syringe filled with the cell suspension at the injection port farthest from the screw valve ports.

4.6. Carefully press the plunger of the syringe to inject the cells **[1-TXT]**.

4.6.1. Talent slowly pushing plunger to deliver cells through the injection port. **TXT: Continue cell injections with the other two ports**

4.7. After all injections, close Ports number 2, 4, and 5, **[1]** and place the MPS device in the 37 degrees Celsius incubator overnight **[2-TXT]**.

4.7.1. Talent closing ports.

4.7.2. Talent placing the device in the incubator. **TXT: Keep the plate level to prevent cells from moving out of the lumen**

Results

5. Results

5.1. The morphology and confluency of isolated PTECs over time are shown in this figure **[1]**. Small patches of PTECs with cobblestone-like morphology emerged by Day 6, interspersed with residual blood cells **[2]**, and formed more defined colonies by Day 8 with reduced blood cell presence **[3]**.

5.1.1. LAB MEDIA: Figure 1.

5.1.2. LAB MEDIA: Figure 1. *Video Editor: Highlight A.*

5.1.3. LAB MEDIA: Figure 1. *Video Editor: Highlight B.*

5.2. By Day 10, cell confluency reached approximately 50 to 60 percent with expanded epithelial colonies **[1]**, and by Day 15, full confluency was achieved across the culture surface **[2]**.

5.2.1. LAB MEDIA: Figure 1. *Video Editor: Highlight C.*

5.2.2. LAB MEDIA: Figure 1. *Video Editor: Highlight D.*

5.3. After injection into the MPS device, PTECs were observed flowing into the circular lumen structure from the injection port, confirming successful cell delivery **[1]**.

5.3.1. LAB MEDIA: Figure 3.

1. **kidney**
   Pronunciation link: https://www.merriam-webster.com/dictionary/kidney Merriam-Webster
   IPA: /ˈkɪd·ni/
   Phonetic Spelling: KID-nee
2. **renal** *(as in "renal capsule", "renal cortex/medulla")*
   Pronunciation link: https://www.merriam-webster.com/dictionary/renal Merriam-Webster+1
   IPA: /ˈri·nəl/
   Phonetic Spelling: REE-nuhl
3. **capsule** *(as in "renal capsule")*
   Pronunciation link: https://www.merriam-webster.com/dictionary/capsule
   IPA: /ˈkæp·səl/
   Phonetic Spelling: KAP-suhl

4. **medulla**
   Pronunciation link: https://www.merriam-webster.com/dictionary/medulla
   IPA: /məˈdʌl·ə/
   Phonetic Spelling: muh-DUL-uh
5. **cortex**
   Pronunciation link: https://www.merriam-webster.com/dictionary/cortex
   IPA: /ˈkɔr·tɛks/
   Phonetic Spelling: KOR-teks
6. **conical** *(as in "conical tube")*
   Pronunciation link: https://www.merriam-webster.com/dictionary/conical Merriam-Webster
   IPA: /ˈkɑ·nɪ·kəl/
   Phonetic Spelling: KON-i-kul
7. **ethanol**
   Pronunciation link: https://www.merriam-webster.com/dictionary/ethanol Merriam-Webster+1
   IPA: /ˈɛθ·əˌnɑl/
   Phonetic Spelling: ETH-uh-nol
8. **slurry**
   Pronunciation link: https://www.merriam-webster.com/dictionary/slurry
   IPA: /ˈslʌr·i/
   Phonetic Spelling: SLUR-ee
9. **centrifuge**
   Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge
   IPA: /ˈsɛn·trəˌfjuʒ/
   Phonetic Spelling: SEN-truh-FYOOJ
10. **tubule** *(as in "proximal tubule epithelial cells")*
    Pronunciation link: https://www.merriam-webster.com/dictionary/tubule
    IPA: /ˈtu·byʊl/
    Phonetic Spelling: TOO-byool
11. **epithelial**
    Pronunciation link: https://www.merriam-webster.com/dictionary/epithelial
    IPA: /ˌɛp·əˈθi·lɪ·əl/
    Phonetic Spelling: ep-uh-THI-lee-uhl
12. **trypsin**
    Pronunciation link: https://www.merriam-webster.com/dictionary/trypsin
    IPA: /ˈtraɪp·sɪn/
    Phonetic Spelling: TRYPS-in
13. **cryovial**
    Pronunciation link: No confirmed link found
    IPA (approximate): /ˈkraɪ·oʊˌvaɪ·əl/
    Phonetic Spelling: CRY-oh-VY-uhl
14. **dimethyl** *(as in "dimethyl sulfoxide")*
    Pronunciation link: https://www.merriam-webster.com/dictionary/dimethyl

IPA: /daɪˈmaɪ·θəl/
Phonetic Spelling: dye-MYE-thuhl

15. **sulfoxide** *(as chemical name suffix)*
Pronunciation link: https://www.merriam-webster.com/dictionary/sulfoxide
IPA: /sʌlˈfɑk·saɪd/ or /sʌlˈfɒk·saɪd/
Phonetic Spelling: sul-FOK-syd