Submission ID #: 67920
Scriptwriter Name: Debopriya Sadhukhan
Project Page Link: https://review.jove.com/account/file-uploader?src=20715648

Title: Development of Human Renal Tubular Epithelial Cell Primary Cultures in Monolayers and Three-Dimensional Conditions

Authors and Affiliations:

Lilian K. Fischer Sigel[1,2], Daiana S. Sánchez[1,2], Alejandro Balestracci[3], Cristina Ibarra[2,4], Claudia Silberstein[1,2]

[1]Laboratorio de Fisiología Renal, Departamento de Ciencias Fisiológicas, Facultad de Ciencias Médicas, Universidad de Buenos Aires (UBA)
[2]Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay), Universidad de Buenos Aires – Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET)
[3]Servicio de Nefrología, Hospital General de Niños Pedro de Elizalde
[4]Laboratorio de Fisiopatogenia, Departamento de Ciencias Fisiológicas, Facultad de Medicina, Universidad de Buenos Aires

Corresponding Authors:

Claudia Silberstein (csilber@fmed.uba.ar)

Email Addresses for All Authors:

Lilian K. Fischer Sigel (kfischersigel@gmail.com)
Daiana S. Sánchez (daaisanchez@gmail.com)
Alejandro Balestracci (abalestracci@yahoo.com.ar)
Cristina Ibarra (cristinaadrianaibarra@gmail.com)
Claudia Silberstein (csilber@fmed.uba.ar)

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: **MM/DD/YYYY**

The video will not be film there. The video has already been filmed in our laboratory in Buenos Aires, Argentina, and the filmmaker (María Victoria Andino) has already sent the filmed fragments for JoVE to edit.

DO NOT use this draft script for filming. Please wait until your script is finalized to begin the filming process.

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

Current Protocol Length

Number of Steps: 19

Number of Shots: 23

Introduction

NOTE to VO: Please record the interview statements

REQUIRED: What is the scope of your research? What questions are you trying to answer?

1.1. Human renal tubular epithelial cells, established in both primary and three-dimensional cultures, were developed as a model to investigate the cytotoxic effects of Shiga toxin, which induces pathophysiological alterations culminating in hemolytic uremic syndrome.

1.1.1. *2.2*

What are the current experimental challenges?

1.2. No specific treatment exists for hemolytic uremic syndrome; therefore, the current challenge lies in evaluating drugs in HRTEC primary cultures to inhibit the effects of Shiga toxin on the human kidney.

1.2.1. *3.7*

What significant findings have you established in your field?

1.3. Shiga toxin has been shown to inhibit cell viability and proliferation, induce apoptosis in HRTEC primary cultures, and suppress the development of tubular structures in three-dimensional HRTEC cultures.

1.3.1. *Figure 3*

What advantage does your protocol offer compared to other techniques?

1.4. The protocols offer the advantage of enabling the study of epithelial cell functional mechanisms in cell cultures that retain the original characteristics of the human renal proximal tubule.

1.4.1. *2.5.1*

Ethics Title Card

The use of human renal tissues for the development of primary cell cultures has been approved by the Ethics Committee of the Hospital General de Niños Pedro de Elizalde

Written informed consent from the guardians on behalf of the children was obtained for the use of kidney samples

Protocol

2. Development of Primary Cultures of Human Renal Tubular Epithelial Cells (HRTEC)

Demonstrator: Lilian K. Fischer Sigel

2.1. To begin, dissect the renal capsule using forceps, separate the renal cortex from the medulla, and cut the cortex into small fragments of approximately 1 millimeter **[1-TXT]**.

2.1.1. LAB MEDIA: CYTEC_008.mp4-h265 02:07-02:26. **TXT: Refrigerate the kidney fragment in PBS or Hanks' solution until processing**

2.2. Place the kidney fragments in sterile Hanks' solution without calcium and magnesium, supplemented with 0.1 percent collagenase type I *(one)* to perform the digestion **[1]**.

2.2.1. LAB MEDIA: CYTEC_011.mp4-h265 00:00-end.

2.3. Incubate the renal fragments for 30 minutes at 37 degrees Celsius with shaking **[1]**.

2.3.1. LAB MEDIA: CYTEC_017.mp4-h265 00:00-end.

2.4. To stop digestion, add one volume of PBS containing 10 percent fetal bovine serum or FBS **[1]**. Wash the sample three times by centrifuging at 160 *g* for 10 minutes each in a centrifuge refrigerated at 4 degrees Celsius, discarding supernatant and resuspending pellet in the same solution each time **[2]**.

2.4.1. LAB MEDIA: CYTEC_019.mp4-h265 00:00-end.

2.4.2. LAB MEDIA: CYTEC_020.mp4-h265 00:00-end.

2.5. Pass the digested product through a 70-micrometer pore mesh to filter the tubules and discard the glomeruli that remain retained in the filter **[1]**.

2.5.1. LAB MEDIA: CYTEC_026.mp4-h265 00:00-end.

2.6. Centrifuge the filtered tubules at 160 *g* for 10 minutes at 4 degrees Celsius **[1]**. After discarding the supernatant, resuspend the pellet in sterile Hanks' solution without calcium and magnesium, supplemented with 0.2 percent collagenase type I *(one)* **[2]**.

2.6.1. LAB MEDIA: CYTEC_020.mp4-h265 00:00-end.

2.6.2. LAB MEDIA: CYTEC_027.mp4-h265 00:00-end

2.7. Incubate the sample for 30 minutes at 37 degrees Celsius with shaking **[1]**.

2.7.1. LAB MEDIA: CYTEC_028.mp4-h265 00:00-end

2.8. To stop digestion, add one volume of PBS containing 10 percent FBS before washing the sample by centrifugation as shown previously **[1-TXT]**.

2.8.1. LAB MEDIA: CYTEC_019.mp4-h265 00:00-end **TXT: Wash the sample 3x by**

centrifugation

2.9. After the last centrifugation, resuspend the cell pellet in 5 milliliters of complete culture medium in a 25 square centimeter culture flask with a vented lid **[1-TXT]**.

2.9.1. LAB MEDIA: CYTEC_033.mp4-h265 00:00-end **TXT: Complete culture medium: RPMI 1640 with 5% FBS, 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 1% endothelial cell growth supplement**

2.10. Close the lid of the culture flask **[1]** and incubate the cells at 37 degrees Celsius in a humid atmosphere with 5 percent carbon dioxide **[2-TXT]**.

2.10.1. LAB MEDIA: CYTEC_034.mp4-h265 00:00-00:08.

2.10.2. LAB MEDIA: CYTEC_034.mp4-h265 00:00-end. **TXT: Next day, wash culture 3× with 5 mL PBS, discard, then add 5 mL complete medium**

2.11. After adding the culture medium and incubating the cells again at 37 degrees Celsius, observe the cells under an inverted microscope **[1-TXT]**.

2.11.1. LAB MEDIA: Figure 1. **TXT: Change the medium with fresh medium every 3 days**

3. Development of Three-Dimensional HRTEC Cultures

3.1. Take an aliquot of liquid basement membrane matrix at a volume of 150 microliters per square centimeter and dispense it into each well of a multi-well plate to cover the bottom **[1-TXT]**. Incubate the plate in a cell culture incubator at 37 degrees Celsius for 1 hour to allow the matrix to solidify into a gel **[2]**.

3.1.1. LAB MEDIA. CYTEC_036.mp4-h265 00:00-00:08. **TXT: Thaw the BM matrix overnight at 4 °C**

3.1.2. LAB MEDIA: CYTEC_037.mp4-h265 00:05-00:16.

3.2. To detach confluent primary HRTEC *(Hrtec)* cultures, after discarding the culture medium from the flask, add 1 milliliter of 0.25 percent trypsin with 0.02 percent EDTA solution **[2]**.

3.2.1. LAB MEDIA.CYTEC_038.mp4-h265 00:00-end.

3.3. Spread the solution evenly across the surface of the flask before incubating it for 5 minutes at 37 degrees Celsius **[1]**.

3.3.1. LAB MEDIA.CYTEC_034.mp4-h265 00:00-00:07.

3.4. Observe the cells under an inverted microscope to confirm complete cell detachment **[1]**.

3.4.1. LAB MEDIA: CYTEC_035.mp4-h265 00:00-00:05.

3.5. After resuspending the detached cells in 10 milliliters of PBS or culture medium, centrifuge at 160 *g* for 10 minutes at 4 degrees Celsius **[1]**.

3.5.1. LAB MEDIA: CYTEC_039.mp4-h265 00:00-end.

3.6. Once the supernatant is discarded and the pellet is resuspended in complete culture medium with the same supplements used for primary HRTEC cultures, count the resuspended cells using a Neubauer chamber **[1]**.

3.6.1. LAB MEDIA: Camara Neubauer(1).jpg

3.7. Seed 40,000 cells in 150 microliters of medium per well onto the solidified basement membrane matrix and incubate the cell cultures for several days at 37 degrees Celsius, in a humid atmosphere with 5 percent carbon dioxide **[1-TXT]**.

3.7.1. LAB MEDIA: CYTEC_037.mp4-h265 00:00-00:17. **TXT: Replace the culture medium with fresh medium every 2 days**

3.8. Monitor the cultures regularly using an inverted optical microscope to observe cell aggregation and formation of tubular structures **[1]**.

3.8.1. LAB MEDIA: CYTEC_042.mp4-h265 00:00-00:05.

Results

4. Results

4.1. HRTEC seeded on the basement membrane matrix **[1]** initiated tubulogenesis by migrating and organizing into multicellular cords within the first 4 to 10 hours of culture **[2]**.

4.1.1. LAB MEDIA: Figure 2.

4.1.2. LAB MEDIA: Figure 2. *Video Editor: Highlight the two images labelled "4h" and "10 h".*

4.2. By 1 day after seeding, cellular aggregates emerged from the cords and began forming early tubular structures **[1]**.

4.2.1. LAB MEDIA: Figure 2. *Video Editor: Highlight the image labeled "1 d".*

4.3. The 3D tubular structures continued to elongate and mature progressively by 3 days and 8 days of culture **[1]**.

4.3.1. LAB MEDIA: Figure 2. *Video Editor: Highlight the two images labelled "3 d" and "8 d".*

4.4. Hematoxylin-eosin staining of the 3D-HRTEC structures revealed a central lumen surrounded by epithelial cells **[1]**.

4.4.1. LAB MEDIA: Figure 3. Video Editor: Highlight the leftmost image labeled "H&E".

4.5. Immunofluorescence confirmed the expression of aquaporin 1 protein in both apical and basolateral membranes of the 3D-HRTEC epithelial cells **[1]**, mirroring the expression pattern observed in proximal tubules of the human kidney **[2]**.

4.5.1. LAB MEDIA: Figure 3. *Video Editor: Highlight the center image labeled "AQP1 in 3D-HRTEC".*

4.5.2. LAB MEDIA: Figure 3. *Video Editor: Highlight the rightmost image labeled "AQP1 in human kidney".*

Pronunciation Guide

1. Renal

- **Link**: — (common word; pronunciation consistent with "reen-uhl")
- **IPA**: /ˈriː.nəl/
- **Phonetic Spelling**: *REE-nuhl*

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2. Cortex

- **Link**: — (commonly used scientific term)
- **IPA**: /ˈkɔːr.tɛks/
- **Phonetic Spelling**: *KOR-teks*

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3. Medulla

- **Link**: — (common term)
- **IPA**: /məˈdʌl.ə/
- **Phonetic Spelling**: *muh-DUL-uh*

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4. Hanks' solution

- **Link**: Cambridge Dictionary (Hank pronunciation) US: /hæŋk/ (How To Pronounce, How To Pronounce, Cambridge Dictionary)
- **IPA**: /hæŋks səˈluːʃən/
- **Phonetic Spelling**: *HANKS soo-LOO-shun*

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5. Collagenase

- **Link**: HowToPronounce pronunciation with phonetics (How To Pronounce)
- **IPA**: /ˌkɒl.əˈdʒeɪ.neɪz/ (British) or /ˌkɑː.ləˈdʒeɪ.neɪz/ (American variant)
- **Phonetic Spelling**: *ah-luh-JAY-nays*

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6. Centrifuge

- **Link**: — (well-known laboratory term)
- **IPA**: /ˈsɛn.trə.fjuːdʒ/
- **Phonetic Spelling**: *SEN-truh-fyooj*

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7. Fetal Bovine Serum (FBS)

- **Link**: — (standard lab abbreviation)
- **IPA**: /ˈfiː.təl ˈboʊ.vaɪn ˈsɪərəm/
- **Phonetic Spelling**: *FEE-tuhl BOH-vyn SEER-uhm*

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8. Trypsin-EDTA

- **Link**: — (common cell culture reagent)
- **IPA**: /ˈtraɪp.sɪn ˌiː.diːˈtiː.eɪ/
- **Phonetic Spelling**: *TRYPS-in EE-dee-TEE-AY*

Trypsin-EDTA /ˈtraɪp.sɪn ˌiː.diːˈtiː.eɪ/ TRYPS-in EE-dee-TEE-AY