Engineering the renal tubule epithelium-endothelial interface

Establishing kidney organoids as a source for proximal tubule epithelial cells

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Introduction

Current **proximal tubule** (PT) drug screening models fall short in recapitulating the human-specific physiological complexity required for successful clinical translatability. To bridge this gap, we are developing a human induced pluripotent stem cell (**hiPSC**)**-derived PT-endothelium interface on chip**. By leveraging hiPSC-derived kidney organoids as a source for hiPSC-derived PT epithelial cells (iPTECs), this model features enhanced cell polarization and robust apical expression of key transporters. Here, the optimization of these organoids as a physiologically relevant cell source for predictive drug safety screening and target discovery is presented.

Methods Overview

- Kidney organoids were derived from hiPSCs as described previously[1].
- iPTECs were sorted by selection with the PT-specific brush-border maker LTL at different time points of the differentiation via MACS[2].

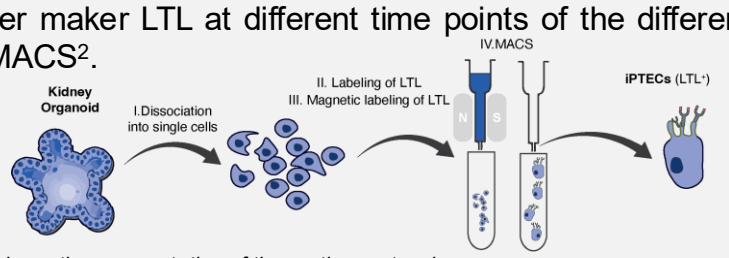


Figure 1: *Schematic representation of the sorting protocol. MACS: magnetic activated cell sorting, LTL: lotus tetraglonobus lectin*

- iPTECs were characterized phenotypically in 2D.
- iPTEC response to different membranes (material and pore size) was analyzed. Permeability of the monolayer was characterized.

Conclusions and Outlook

- Kidney organoids serve as a **viable source for iPTECs** through MACS isolation targeting the brush border marker LTL. The maximum yield is achieved at day 28 (*specific to this hiPSC line and differentiation protocol).
- Freshly isolated iPTECs show **PT-specific marker expression**. However, consistent with the 3D-to-2D transition, undergo a loss of certain PT markers upon sorting.
- Comparative **RNA-seq** analysis of intact organoids *vs.* freshly sorted iPTECs and 2D monolayers will help to identify differentially expressed genes and downregulated metabolic pathways, providing a roadmap for targeted rescue of the PT phenotype, while verifying cell identity.
- Future efforts will focus on recapitulating physiological cues, including shear stress, permeable substrates, and endothelial co-culture, to restore **3D architectural organization** and PT marker expression within a microfluidic chip.

References

1. Morizane R., *et al.* Nat Biotechnol **33** (11):1193-200 (2015). DOI: 10.1038/nbt.3392.
2. Ma, C., *et al.* iScience **27** (33): 110780 (2025). DOI: 10.1016/j.isci.2024.110760

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Results and Discussion

Addressing the inherent variability of hiPSC-derived kidney organoids, a **longitudinal study to characterize PT development** and **maximize iPTEC sorting efficiency** was performed. Kidney organoids exhibit varying proportions of PTECs (LTL$^+$, green), peaking at D28. Sorting yields the higher iPTEC recovery for days 21 and 28 (from ~250 organoids). Post-sorting analysis confirms the enrichment of iPTECs in the positively sorted fraction.

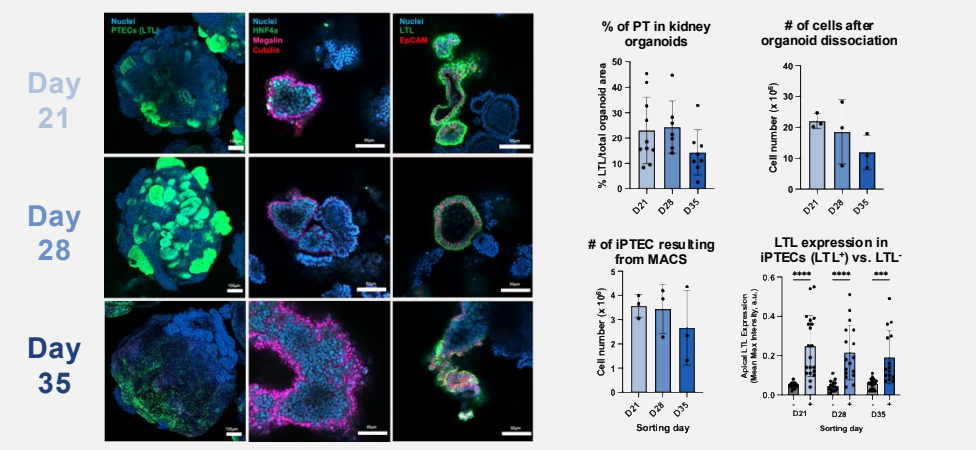


Figure 2: *Immunophenotyping confirms that resident PT cells express HNF4a (transcription factor), megalin and cubilin (apical endocytic transporters), and EpCAM (epithelial junction protein), while remaining negative for the distal tubule marker E-Cadherin. N=3, with batches of organoids independently differentiated from hiPSCs. N > 15 cells for LTL expression.*

The **expression of PTEC-specific markers** was evaluated at three distinct stages: 24 hours post-isolation, upon reaching confluency, and following serial passaging.

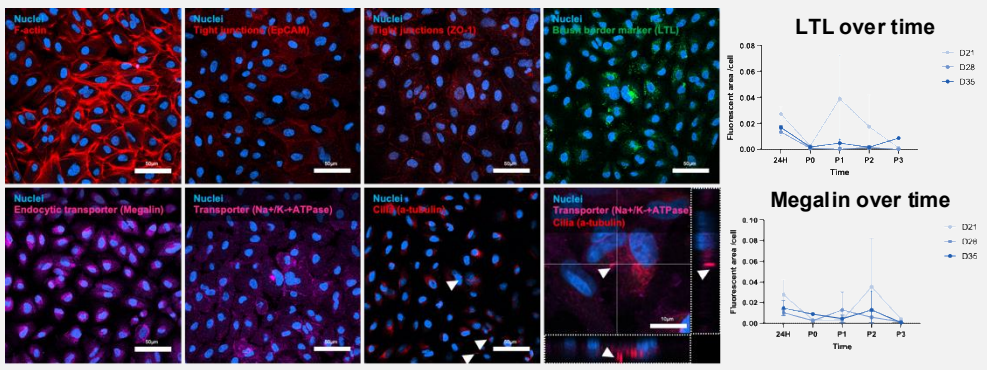


Figure 3: *Day 28-derived iPTECs exhibit a characteristic epithelial cobblestone morphology, with EpCAM and ZO-1 defined tight junctions. The cells retain key PT identity markers, including an apical brush border and the endocytic transporter megalin. Distinct apico-basal polarization is evidenced by the basolateral localization of the Na$^+$/K$^+$-ATPase pump and the presence of primary cilia. Notably, the expression of megalin and the LTL+ brush border progressively decline over time and upon subsequent passaging, indicating a loss of phenotypic stability in extended 2D culture.*

To evaluate the suitability of iPTECs for **functional transport assays**, their capacity to form leak-tight monolayers across a variety of semi-permeable membranes was characterized. iPTECs form a tight monolayer on 0.4 µm pore-size polycarbonate (PC) and polyester (PET) membranes.

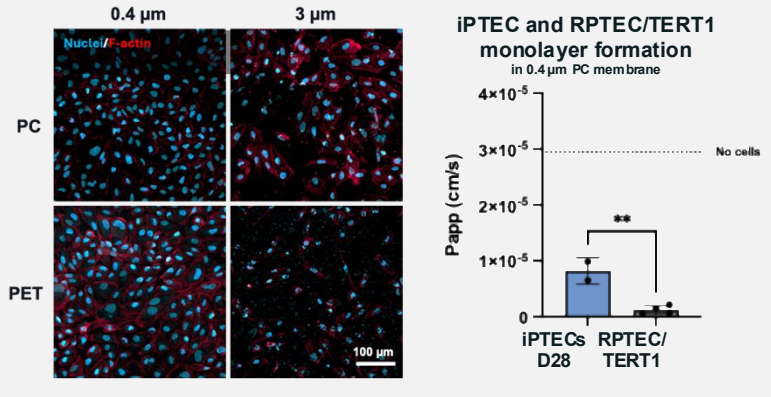


Figure 4: *Monolayer formation is compromised on 3.0-µm-pore membranes regardless of material, indicating that smaller pore diameters are required for epithelial barrier maturation. N>2, where each dot represents the average of three technical replicates.* Ex. 485/20, Em. 516/20

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