CO-AXIAL BIOPRINTING OF CONVOLUTED PROXIMAL TUBULE FOR KIDNEY DISEASE MODELING

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STATE OF THE ART

Metabolic conditions, genetic defects or drug toxicity can cause damage to the **proximal tubule** (PT) leading to kidney failure. More representative biomimetic ***in vitro* models** are needed to gain insight in these processes. PT epithelial cells (PTECs) and genetic manipulations (**conditional immortalization, CRISPR-Cas9**) allow to create cell models representative for diseased stages. Further, 3D (bio) printing offers new modelling alternatives to incorporate extracellular matrix (ECM) interactions and native-like curvature, both fundamental for the correct behaviour of PTECs.

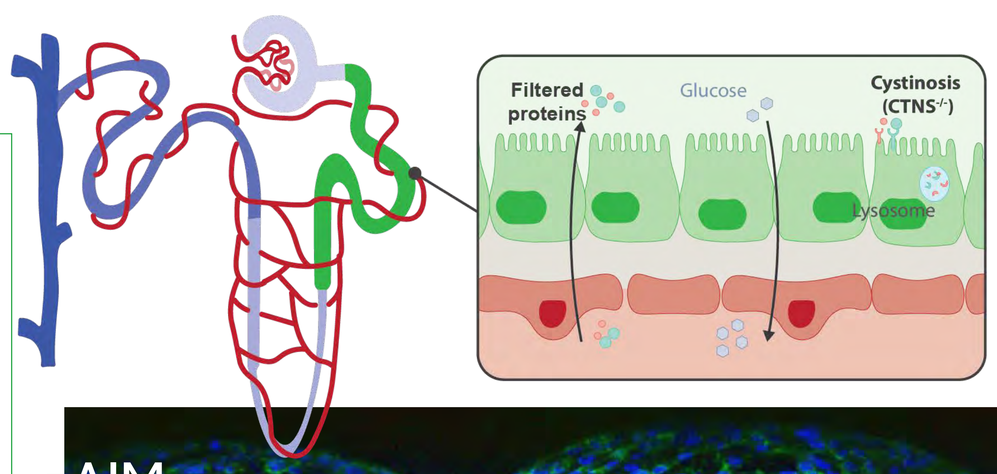


Figure 1. The proximal tubule, main site of renal reabsorption and secretion processes. In cystinosis (*CTNS*$^{-/-}$), lysosomal accumulation of cysteine leads to PT integrity loss.

AIM

Apply **extrusion coaxial (bio) printing** to create a hollow convoluted channel within a gelatin-based microfiber to model the convoluted structure of the PT for healthy and cystinosin-deficient (*CTNS*$^{-/-}$)[1,2] PTECs.

METHODS

1. Development of a coaxial bioprinting system and a biocompatible and stable biomaterial ink

- Gelatin 8%
- Alginate 1%
- Microbial transglutaminase 2%
- $CaCl_2$ 2%

2. Microfiber fabrication and seeding

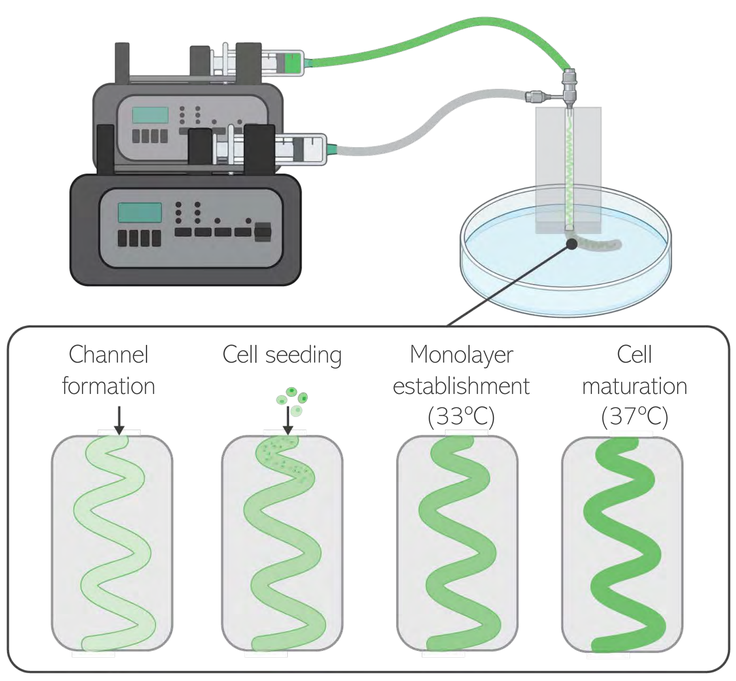


Figure 2. Schematic representation of the 3D(bio)printing setup and biofabrication steps

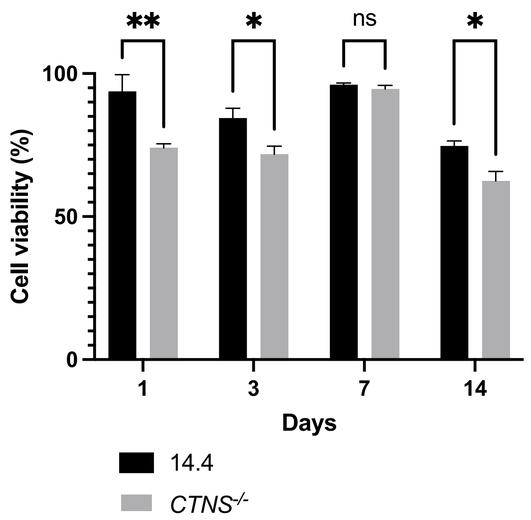
3. Assessment of cell viability (calcein-AM, Ethidium Homodimer-1)

4. Immunofluorescent stainings for cytoskeleton organization (F-actin) and barrier formation (inulin-FITC leakage)

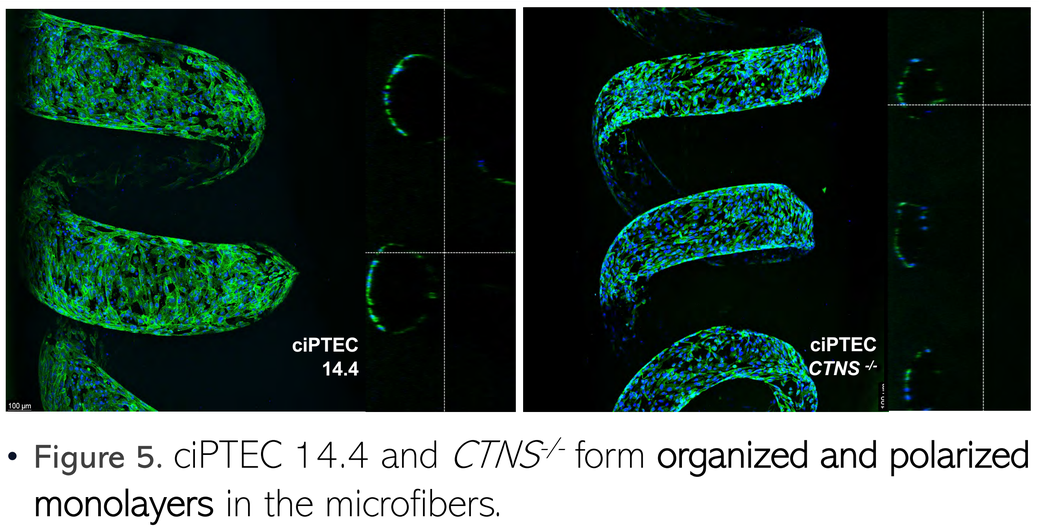
5. Gene expression analysis (qPCR)

6. Membrane transporter activity using calcein-AM and inhibitors for apical efflux pumps MRP4 and BCRP

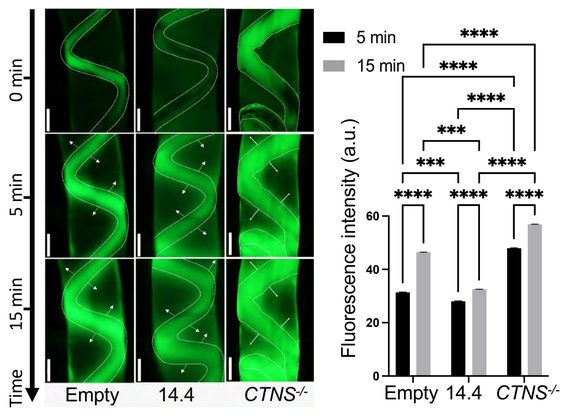
RESULTS



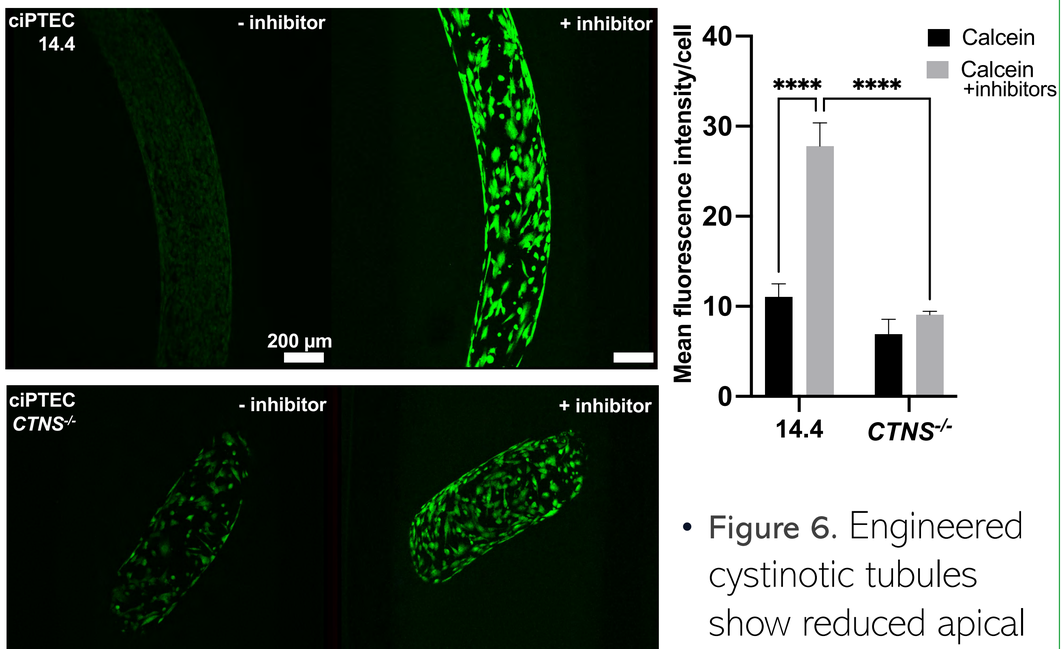
- **Figure 3.** High cell viability within the first week of culture. Convoluted cystinosis tubules are less viable upon prolonged culturing.



- **Figure 5.** ciPTEC 14.4 and *CTNS*$^{-/-}$ form **organized and polarized monolayers** in the microfibers.



- **Figure 4.** Healthy monolayers are **tight** whilst diseased are **leaky.**



- **Figure 6.** Engineered cystinotic tubules show reduced apical transport activity.

CONCLUSION

Our printing system allows the fabrication of coiled microfibers replicating healthy and diseased PTs. This mimics physiologically relevant 3D microenvironment in which cells organize in monolayers and exhibit mature makers (polarization, apical transporters).

References:

1. Wilmer, M.J., et al. "Novel conditionally immortalized human proximal tubule cell line expressing functional influx and efflux transporters." Cell and tissue research 339.2 (2010): 449-457.
2. Jamalpoor, A., et al. "Cysteamine–bicalutamide combination therapy corrects proximal tubule phenotype in cystinosis." EMBO Molecular Medicine 13.7 (2021): e13067.

Acknowledgements:

Dutch Kidney Foundation (17PHD16, 18KVP01), Utrecht Institute for Pharmaceutical Sciences, Materials Driven Regeneration Young Talent Grant, Hofvijverkring Visiting Scientist Program, Brigham Research Institute