Advanced *in vitro* models for drug development: the complexity of simplicity

Roos Masereeuw, *div. Pharmacology,*
Dept. Pharmaceutical Sciences, UU, NL

***U**trecht*
***I**nstitute for*
***P**harmaceutical*
***S**ciences*

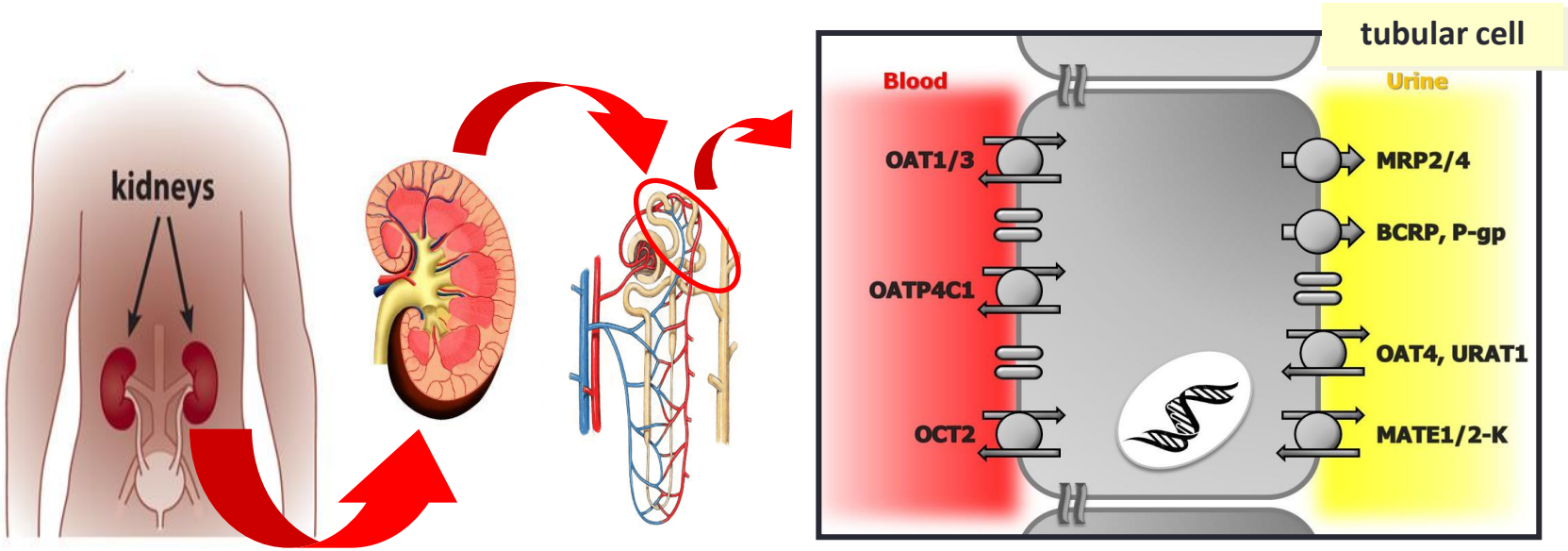
Universiteit Utrecht

Research at experimental pharmacology

- Tools for novel therapeutic strategies to increase organ function during disease

- Gain insight in processes that determine renal excretion of metabolic wastes and drugs to develop interventions at end stage kidney disease

The kidney and its functions



Kidneys

Regulate:
Body fluid volume
Blood pressure
pH (acid-base homeostasis)
Osmolarity

Hormone secretion/regulation:
Erythropoietin
Calcitriol
RAAS
Vitamin D

Nutrient reabsorption

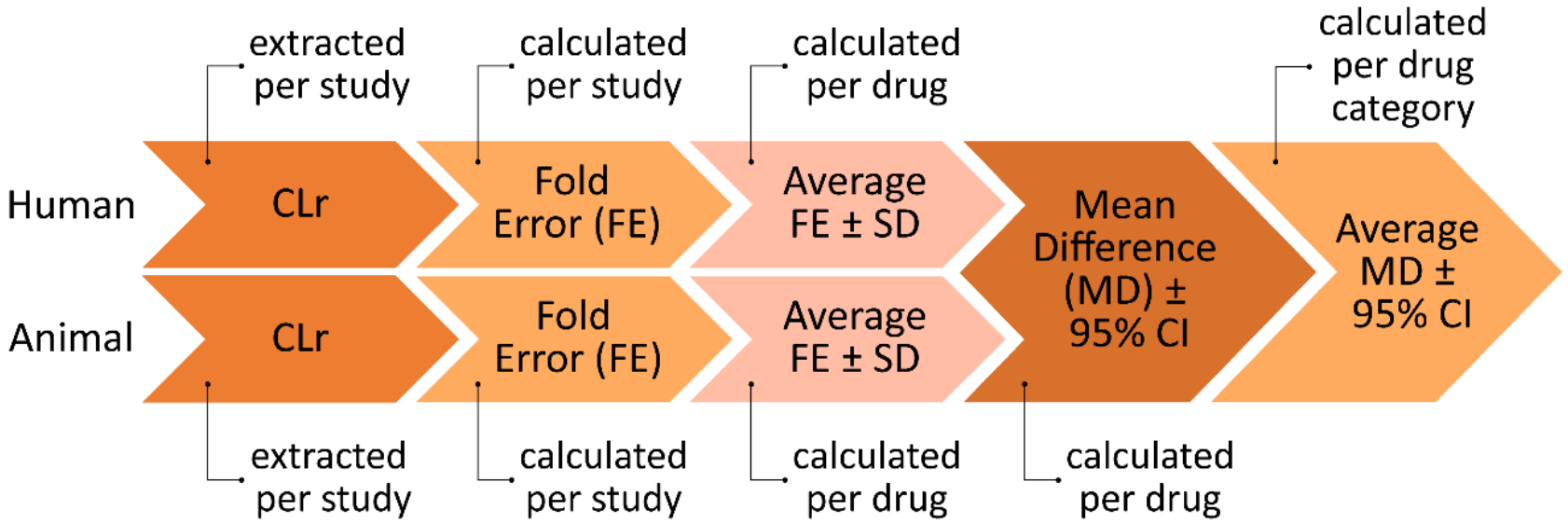
Excretion
Metabolic waste
Exogenous substances

Renal drug handling: translational challenges

Artwork ‘Youngman’ by Tim Noble and Sue Webster, 2012

Humans are animals, but are animals human enough?

- Allometric scaling is suitable for prediction of human renal drug clearance (CLr)

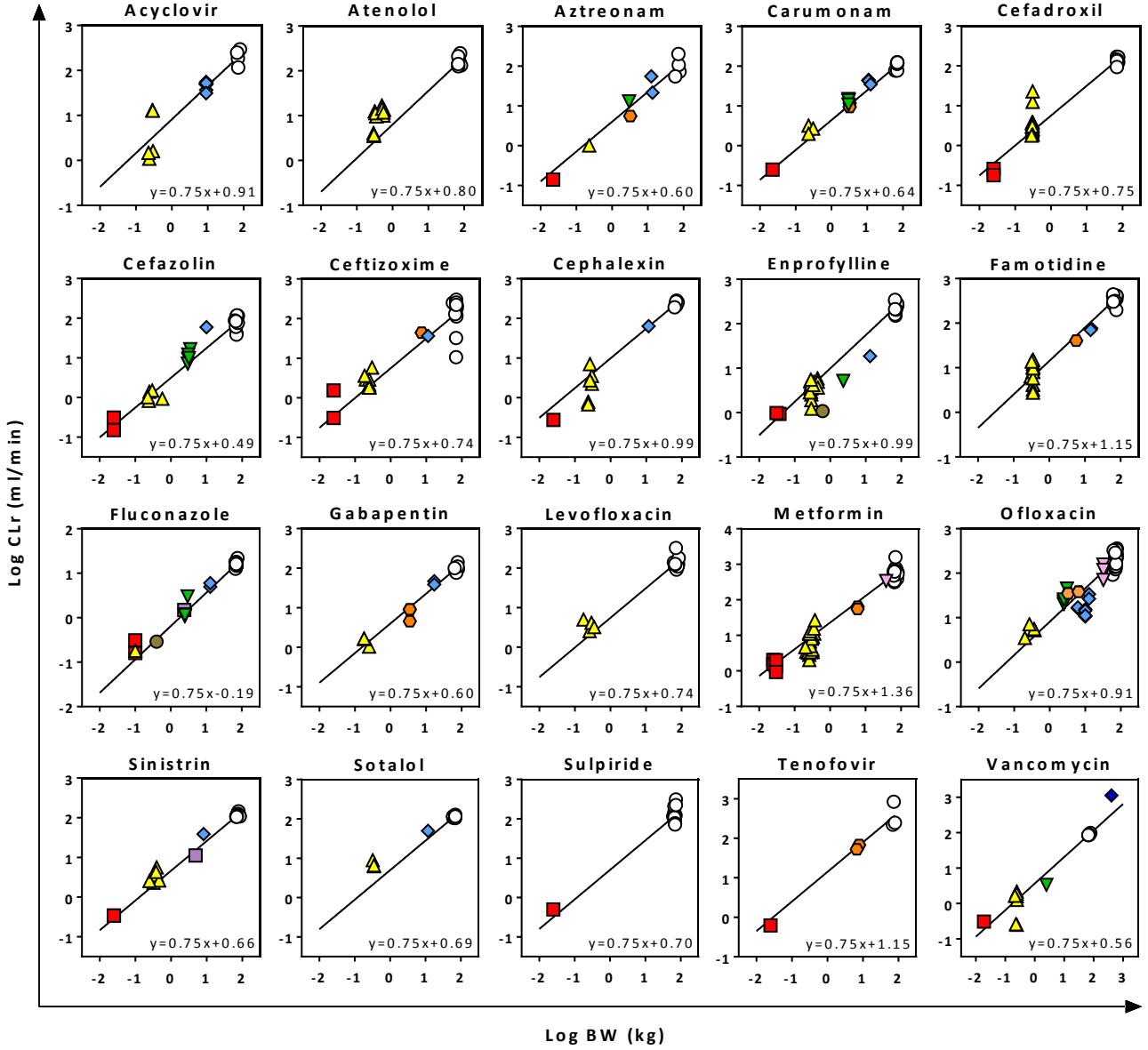


- The average CLr of a diverse set of 20 drugs scales to the 3/4 power of body mass

Universiteit Utrecht

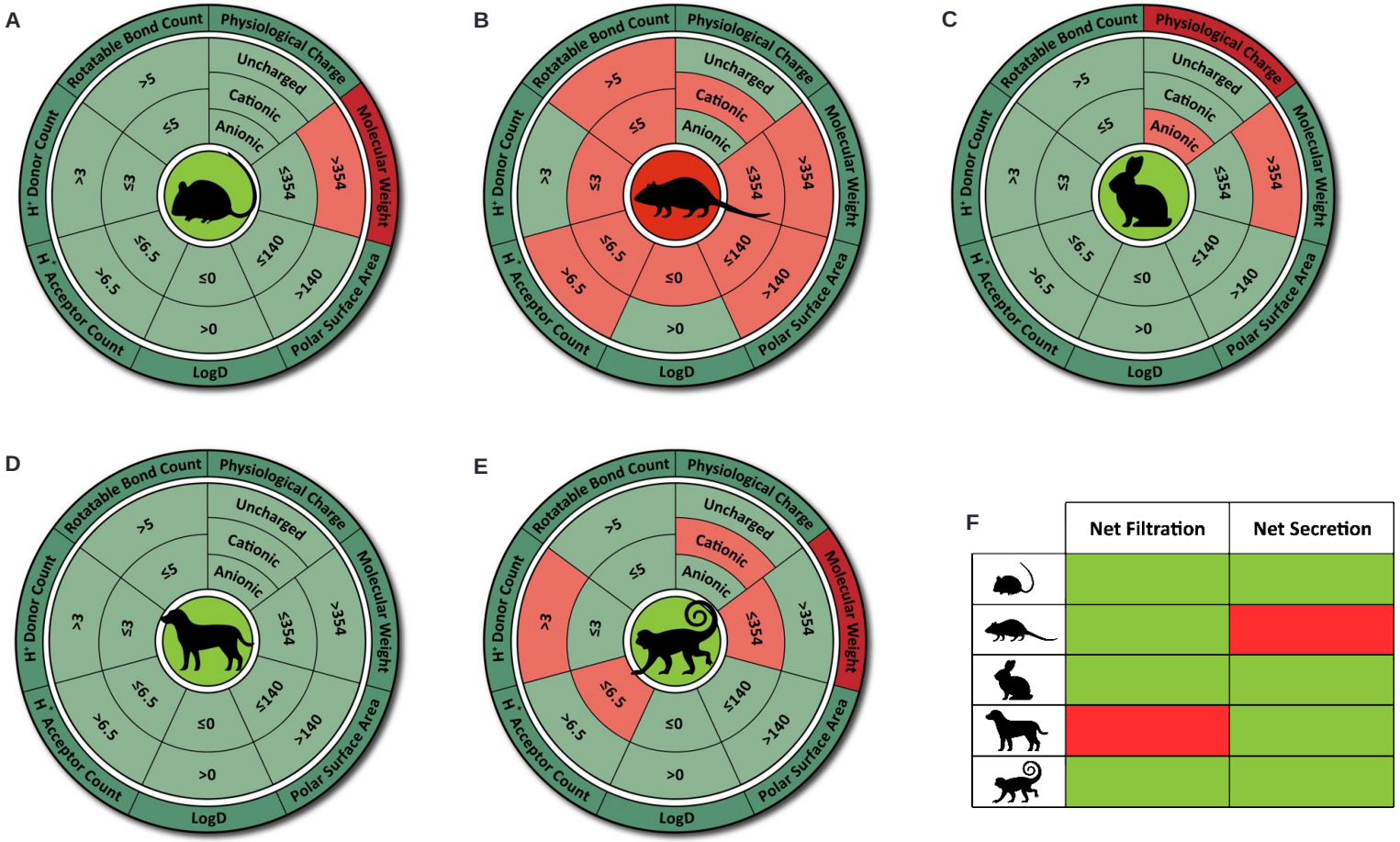
Jansen, K., Pou Casellas, C., *et al.*
Drug Discovery Today 2020

Humans are animals, but are animals human enough?



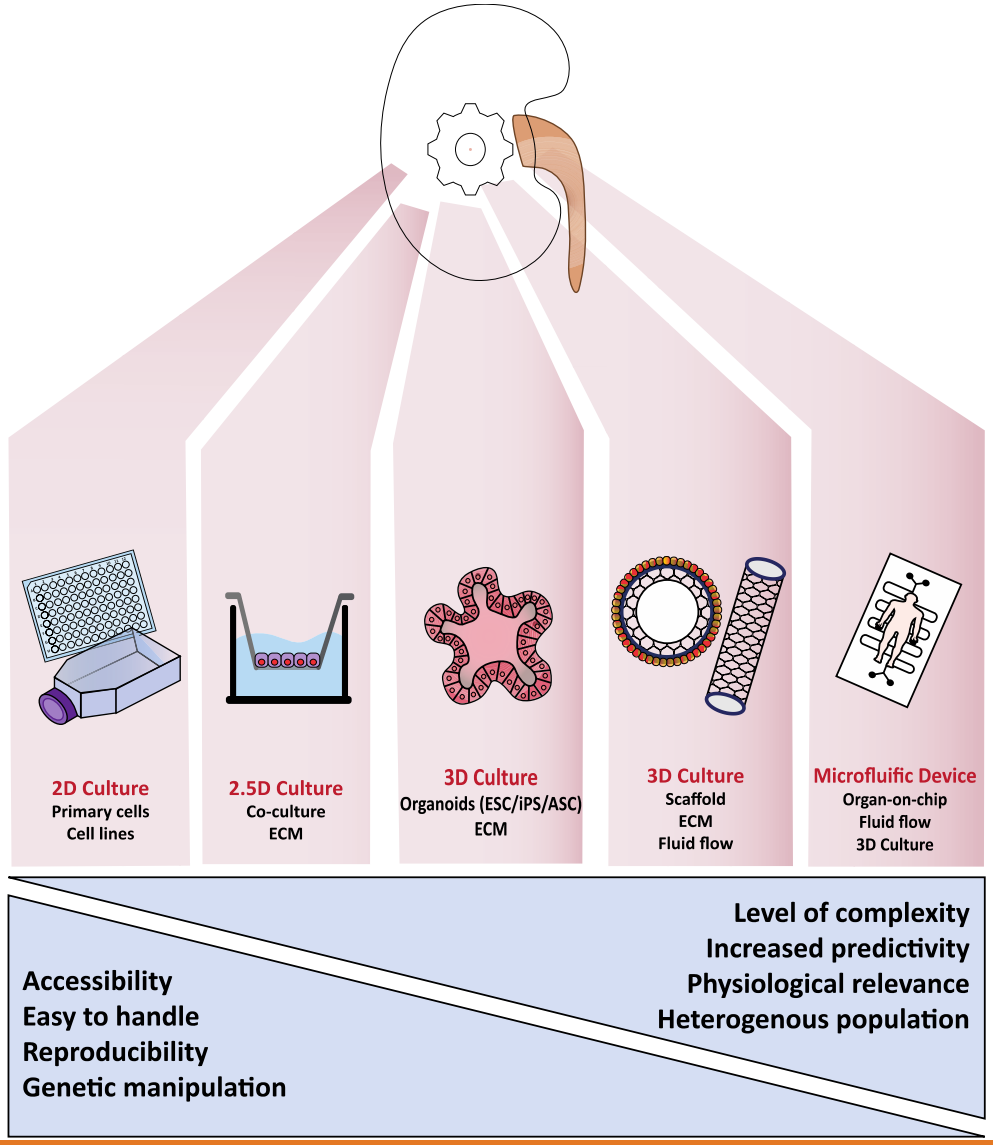
Universiteit

Humans are animals, but are animals human enough?



- Rat models should be used with caution for drug disposition studies
- Meta-analyses of (pre)clinical data can reduce PK animal experiments

Renal drug handling: predictional challenges

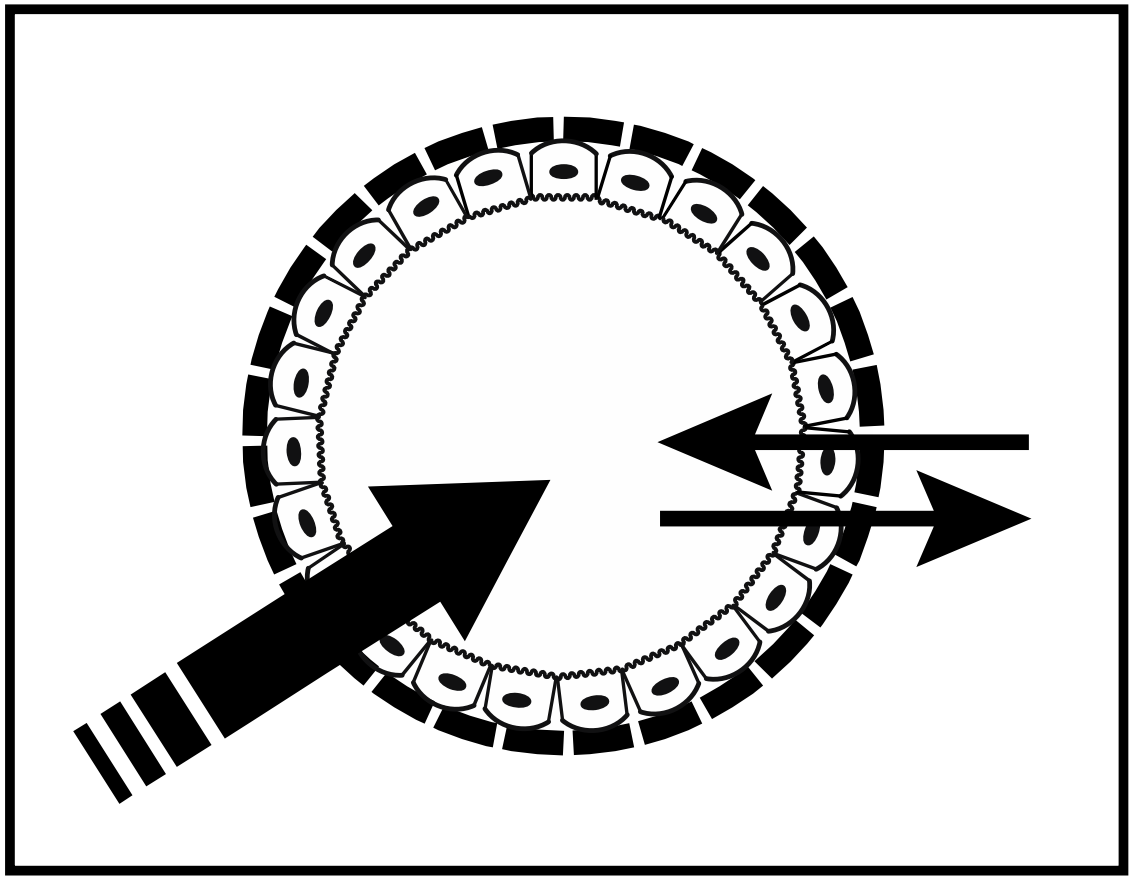


- **Increasing complexity** reduces reproducibility and through-put analysis

- **Increasing complexity** increases predictivity and physiological relevance, but also costs and manipulation

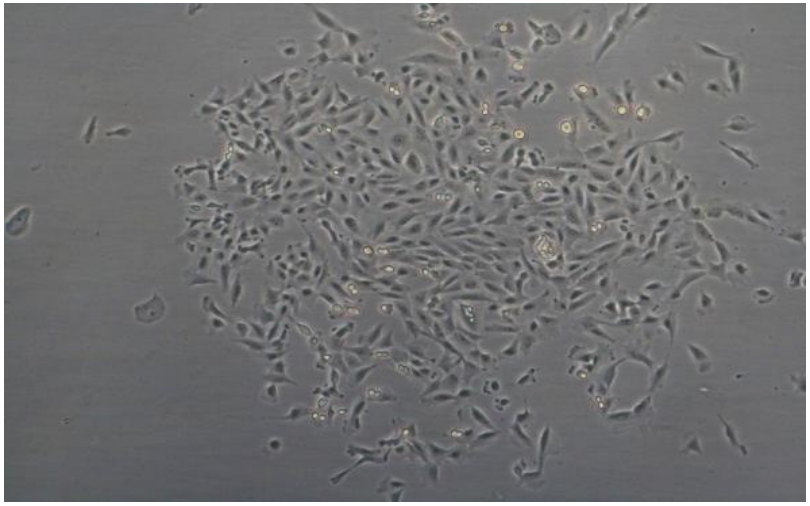
Faria, J., Ahmed, S. *et al.* Arch. Toxicol. 2019

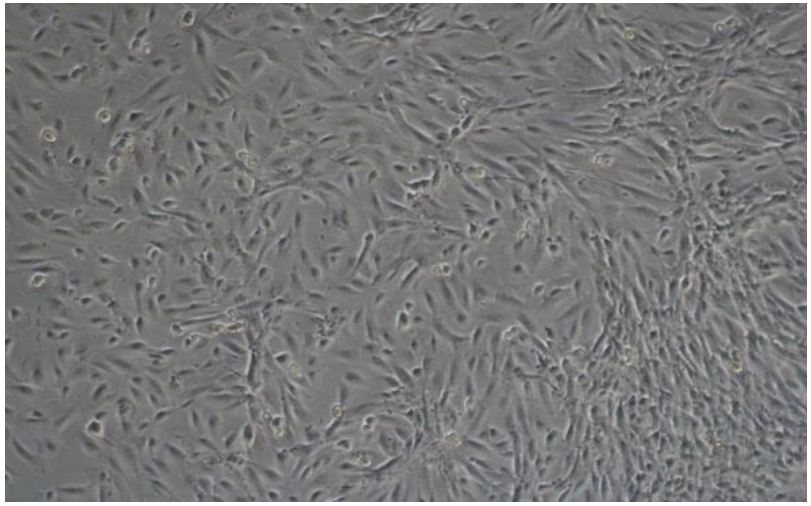
Advanced *in vitro* models: bioengineered kidney tubules



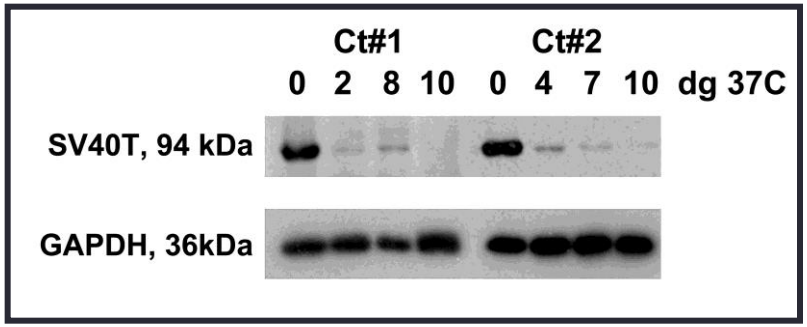
Bioengineering kidney tubules

Conditionally Immortalized Proximal Tubular Epithelial Cell (ciPTEC)





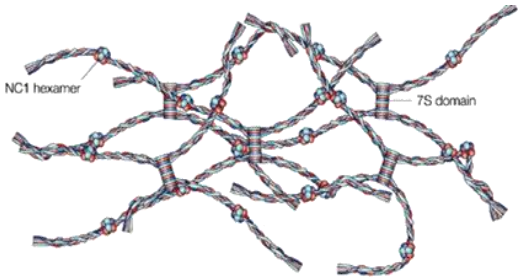
- Immortalization:
  1. SV40T tsA58 U19
  2. hTERT



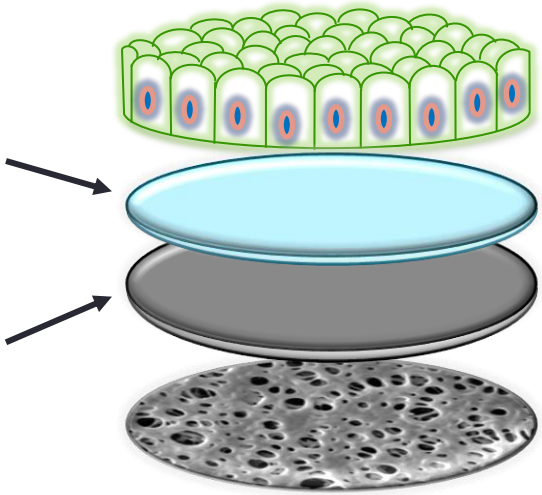
Wilmer *et al.* Cell Tissue Research 2010

Bioengineering kidney tubules: membranes

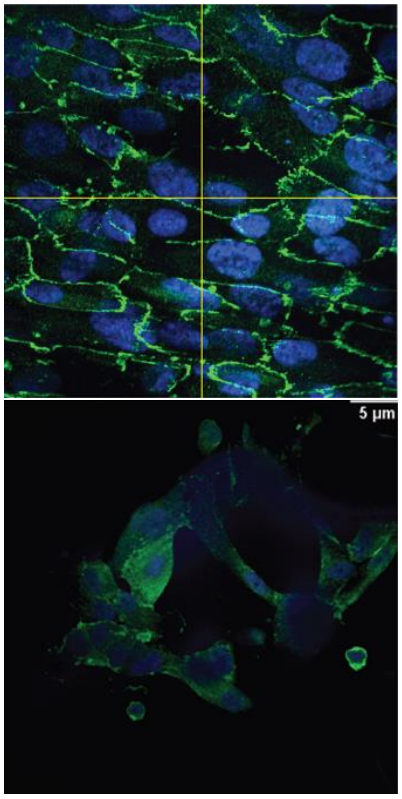
Collagen IV



L-Dopa



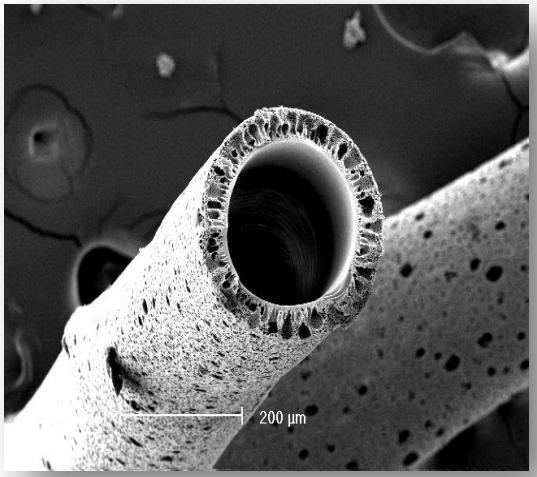
Coated membrane



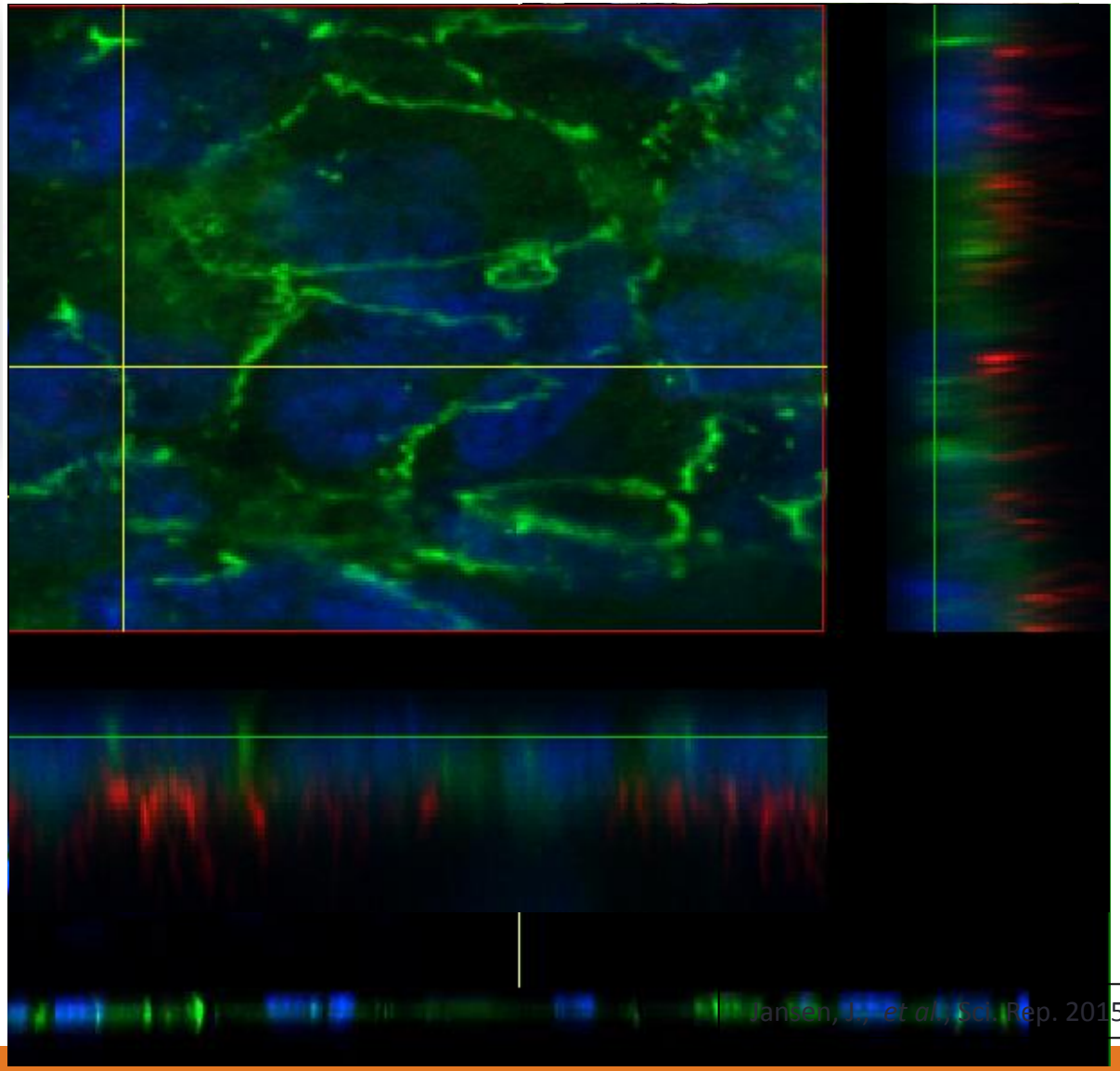
Uncoated membrane

Schophuizen, *et al*, *Acta Biomaterialia*, 2015

Bioengineering kidney tubules

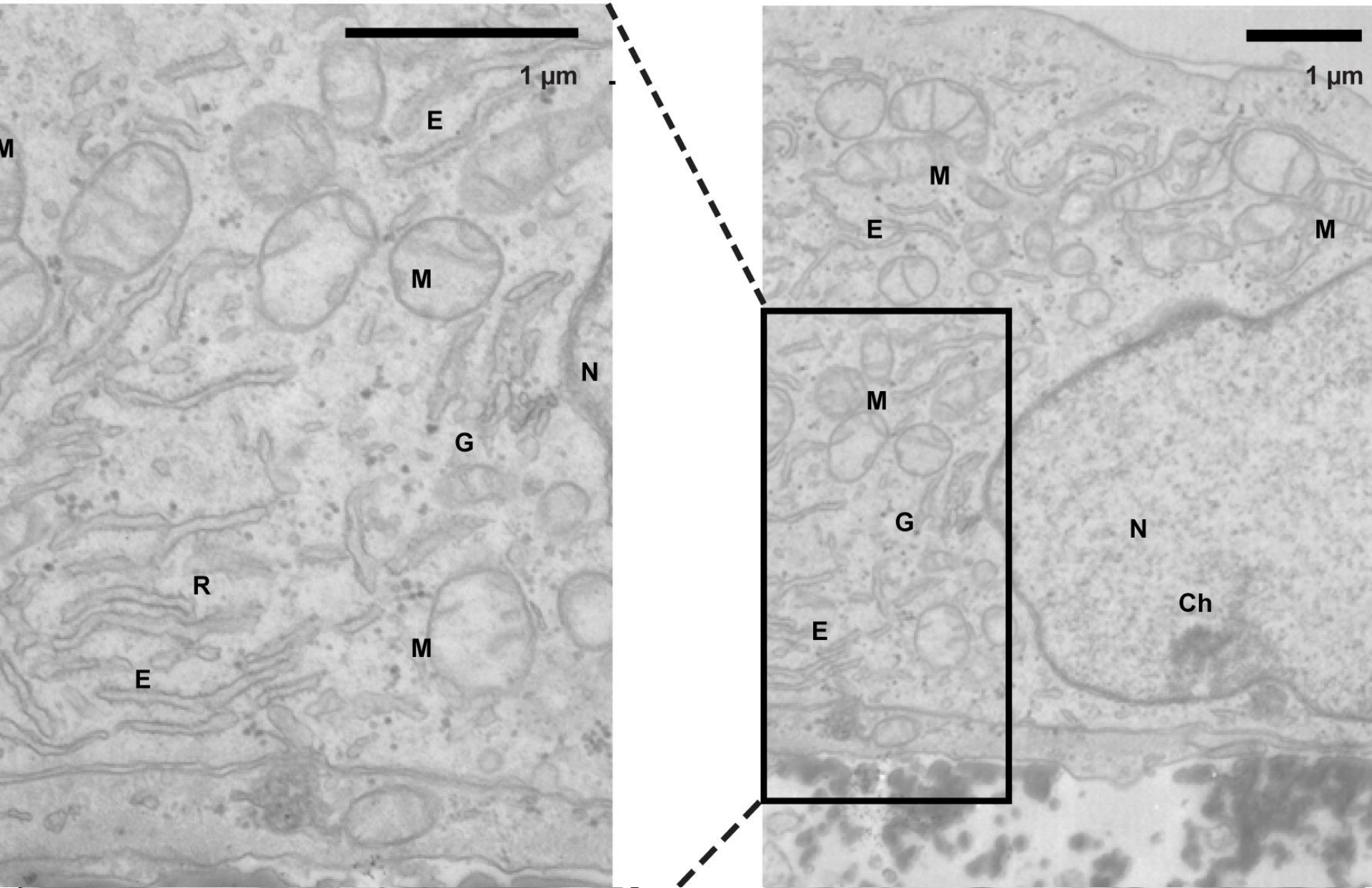


- ZO-1
- Nucleus
- OCT2

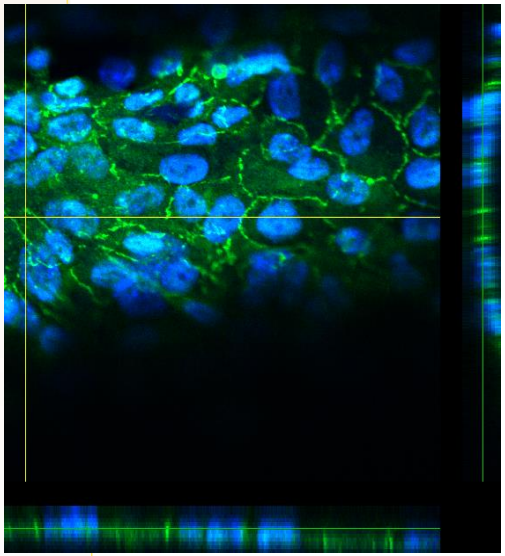


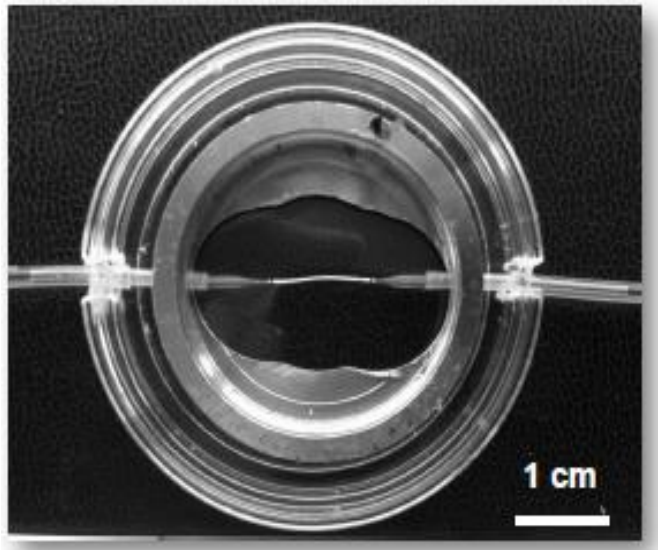
Janben, U, *et al*, Sci. Rep. 2015

Bioengineered kidney tubules



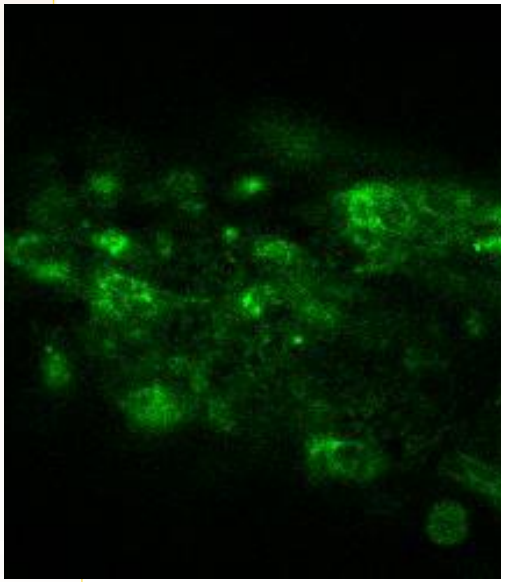
Functional imaging of bioengineered kidney tubules

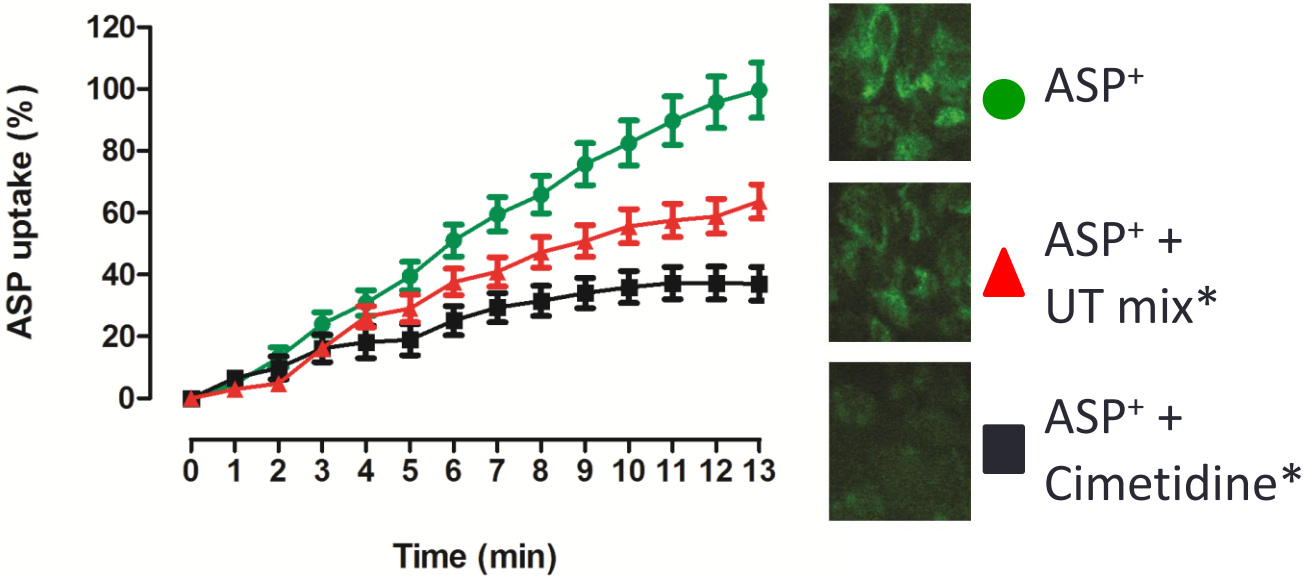




Jansen, J., *et al.*, Sci. Rep. 2015

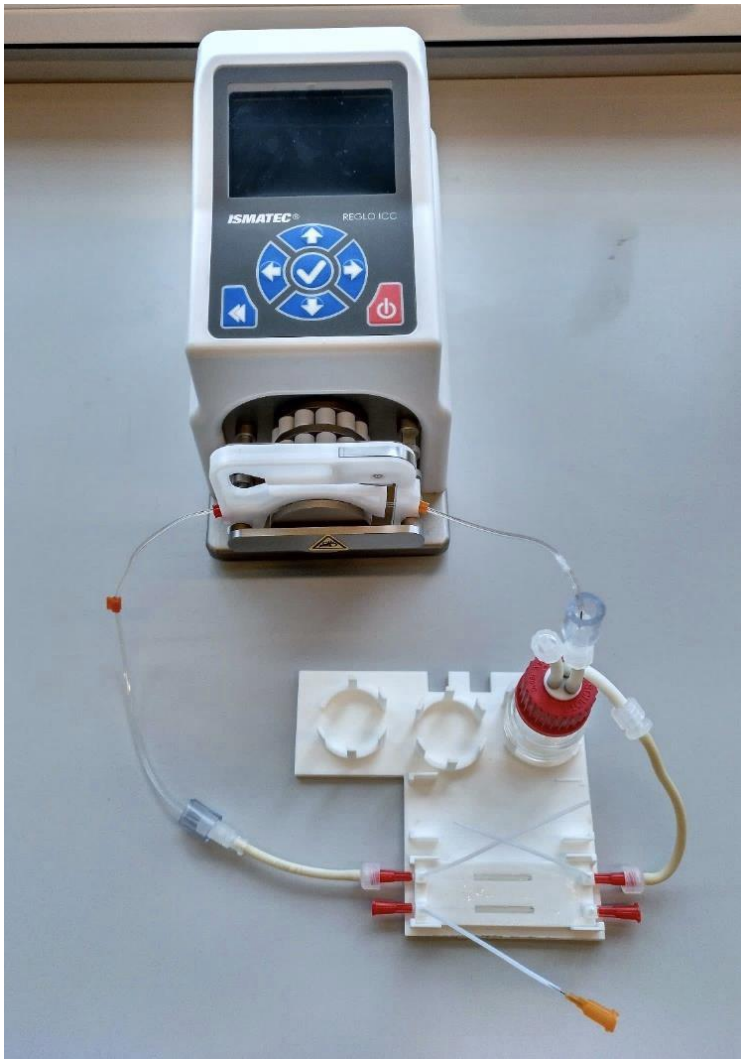
Functional imaging of bioengineered kidney tubules

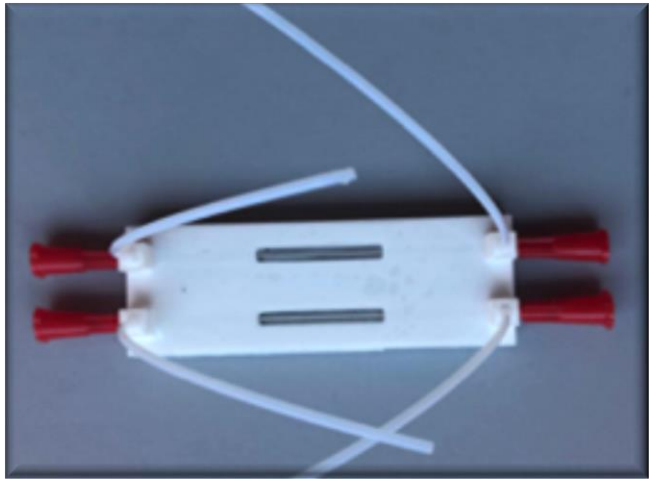




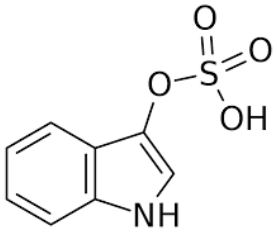
Jansen, J., *et al.*, Sci. Rep. 2015

Bioengineered kidney tubules in microfluidics

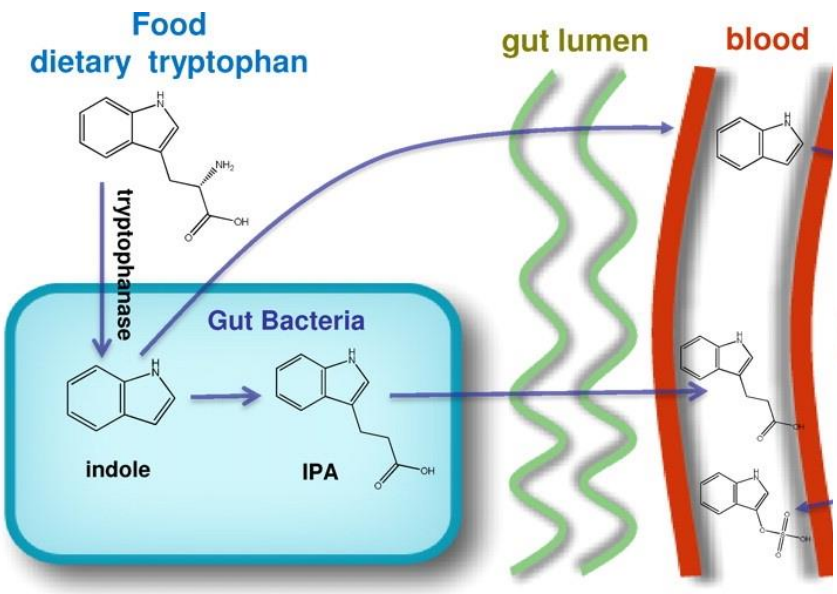


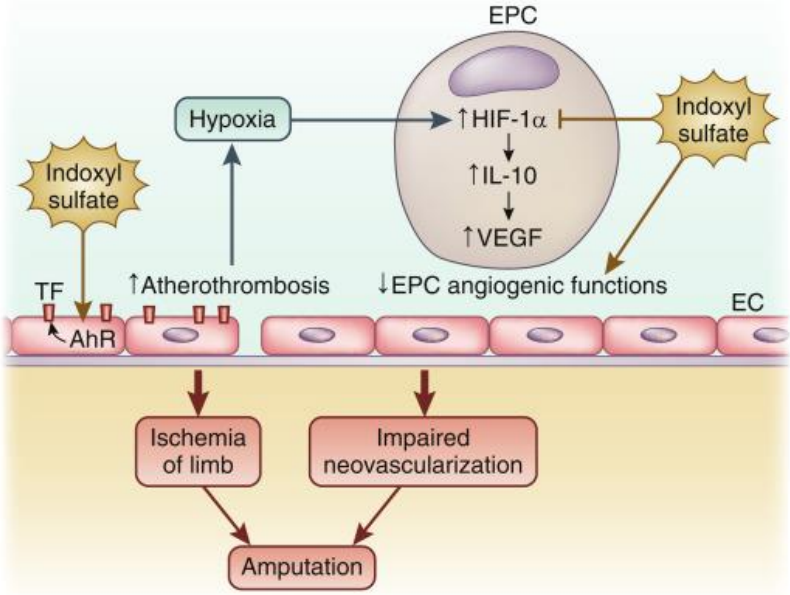


Bioengineered kidney tubules: indoxyl sulfate excretion



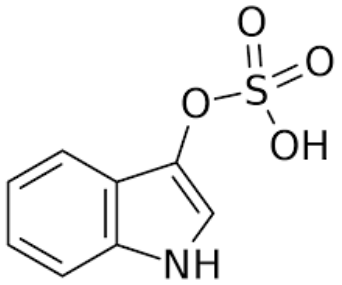
Indoxyl Sulfate



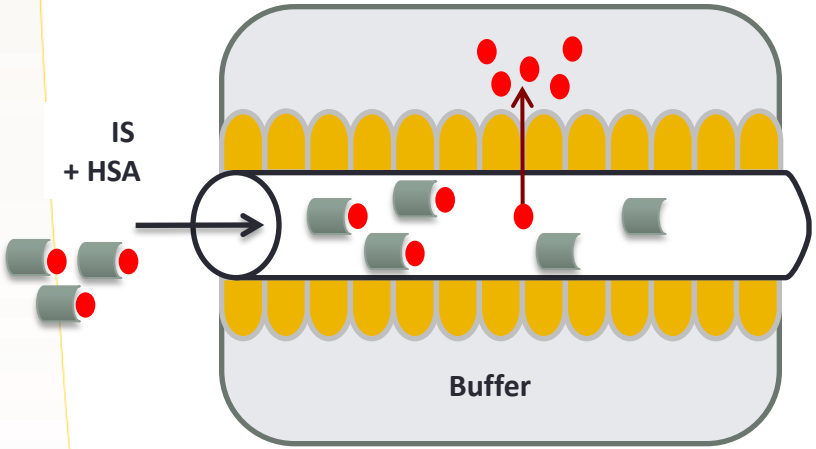


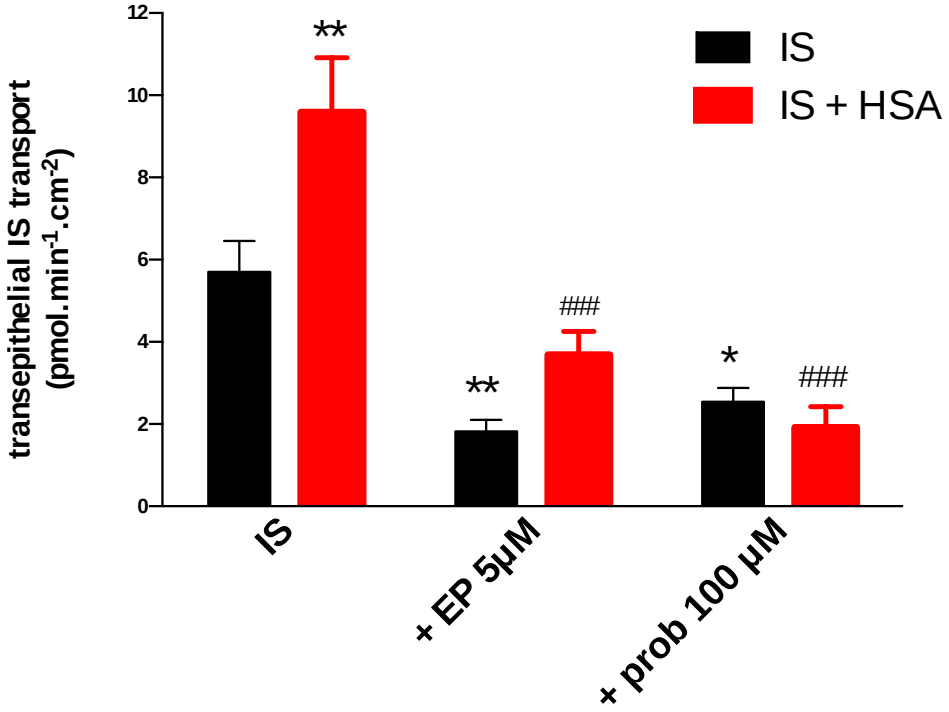
Meijers & Evenepoel, NDT. 2011; Dou & Burtey, Kidney Int. 2016

Bioengineered kidney tubules: indoxyl sulfate excretion



Indoxyl Sulfate

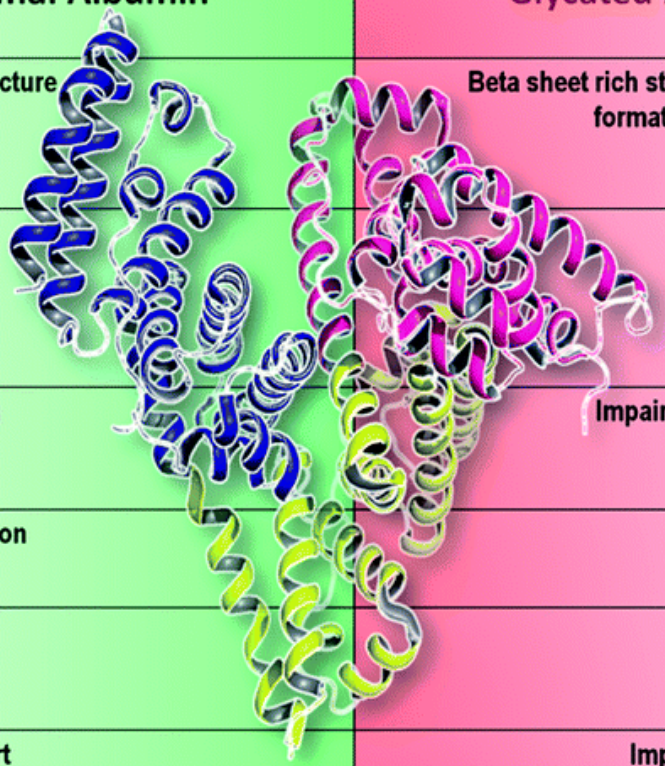




Universiteit Utrecht

Jansen ,J., Fedecostante, M., *et al*. Sci. Rep. 2016

Bioengineered kidney tubules: indoxyl sulfate excretion



Normal Albumin	Glycated Albumin	Reference
Native helical structure	Beta sheet rich structure, aggregation and formation of cross beta sheets.	Bouma et al. 2003, Obrenovich and Monnier 2004.
Drug binding	Impaired drug binding properties	Baraka-Vidot et al. 2012, Vetter and Indurthi, 2013, Awasthi et al. 2015b.
Fatty acid binding	Impaired fatty acid binding and transport	Yamazaki et al. 2005, Blache et al. 2015.
Antioxidant function	Loss of antioxidant function	Bourdon et al. 1999, Faure et al. 2008.
Esterase function	Loss of esterase activity	Ahmed et al. 2005, Baraka-Vidot et al. 2015.
Hormone transport	Impaired hormone transport	Watanabe et al. 1991, Petitpas et al. 2003.
Protective effect on cells (Endothelial cells, RBCs, Muscle cells etc.)	Deleterious effect on cells (cytotoxicity, inflammation, and membrane damage)	Amore et al. 1997, Hattori et al 2001, Awasthi et al 2015.

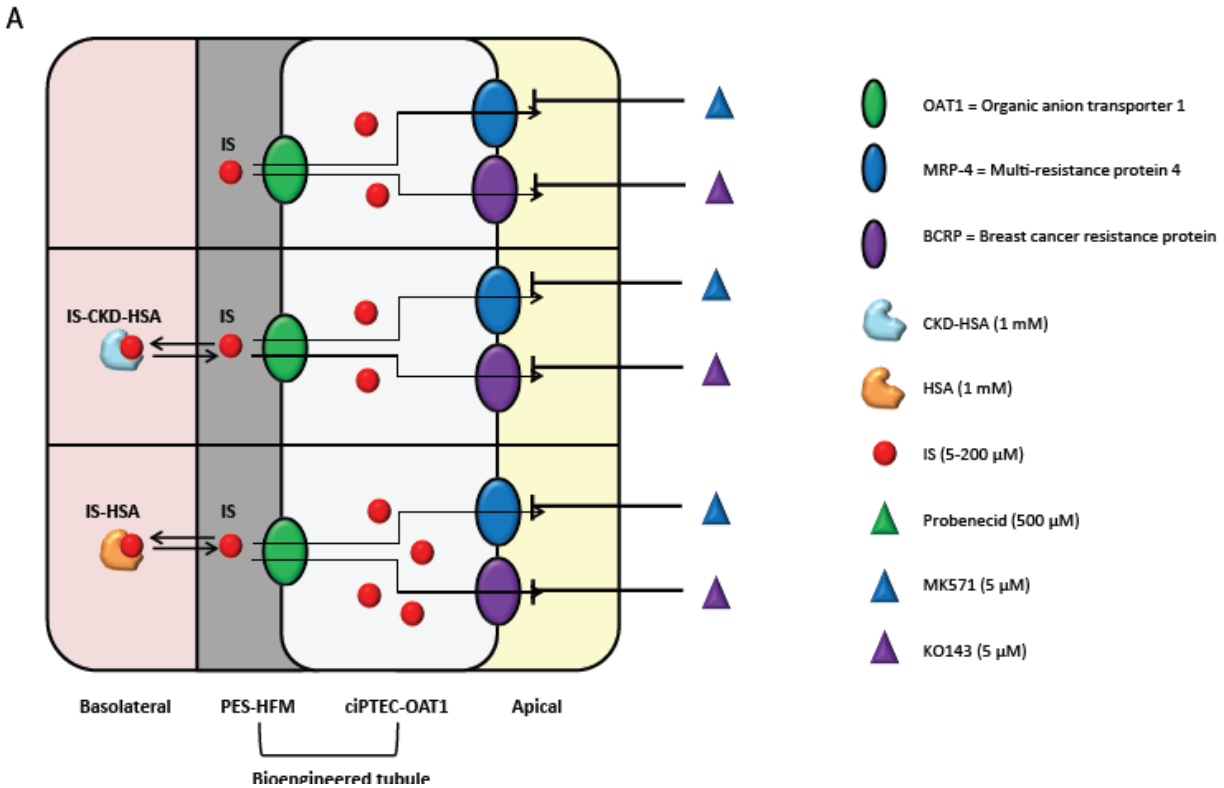
Awasthi and Saraswathi, RSC Adv., 2016

Bioengineered kidney tubules: indoxyl sulfate excretion

Free IS

CKD-HSA IS

HSA IS

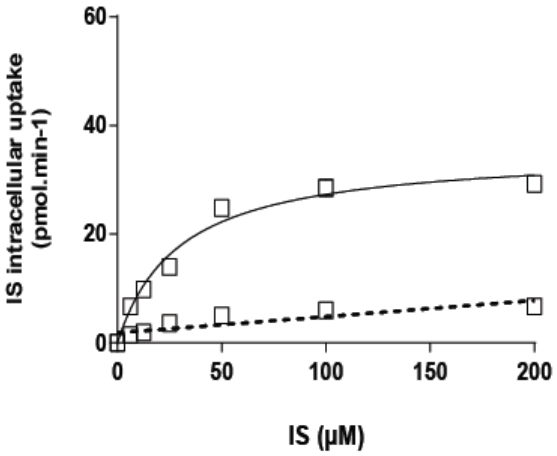


Universiteit Utrecht

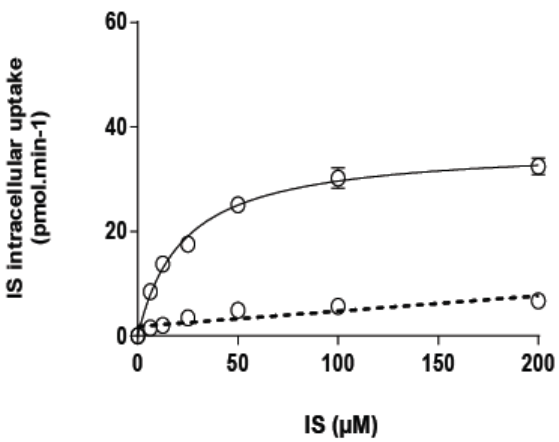
Van der Made, T., *et al*. Mol. Pharm. 2019

Bioengineered kidney tubules: indoxyl sulfate excretion

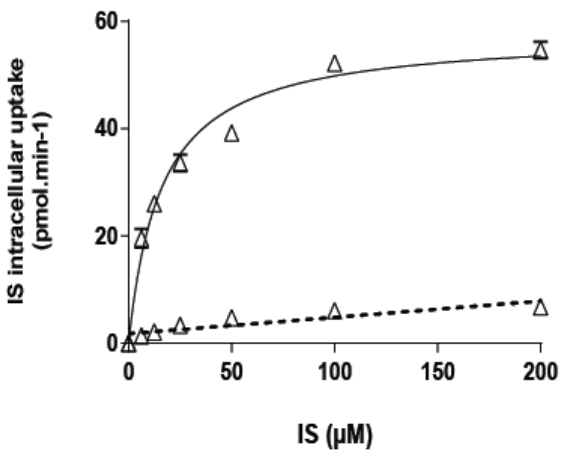
Free IS



CKD-HSA IS



HSA IS

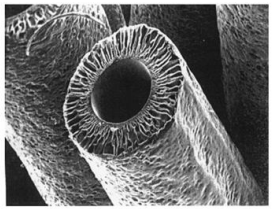


	Free IS	CKD-IS	HSA-IS
K_m (µM)	29.3	5.4	1.4
$CL_{int,u}$ (µL/min/10^6 cells)	0.90	5.1	33.7

Albumin supports renal secretion of drugs and metabolic wastes

Van der Made, T., *et al*. Mol. Pharm. 2019

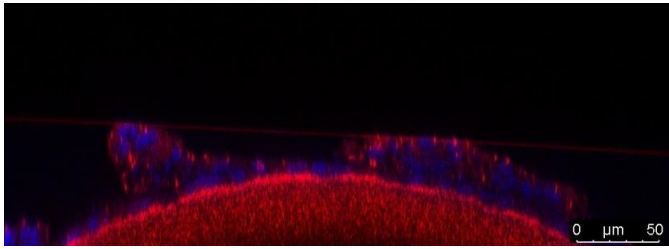
Bioengineered intestinal tubules

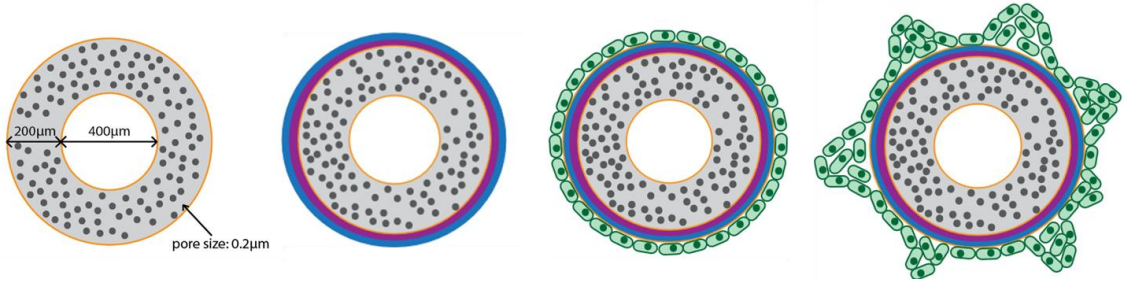


Extracellular Matrix

14 days Static

7 days flow





Epithelial barrier

Main small intestinal cell types present

Villi-like formation

Toxicology in Vitro 60 (2019) 1–11

Contents lists available at ScienceDirect

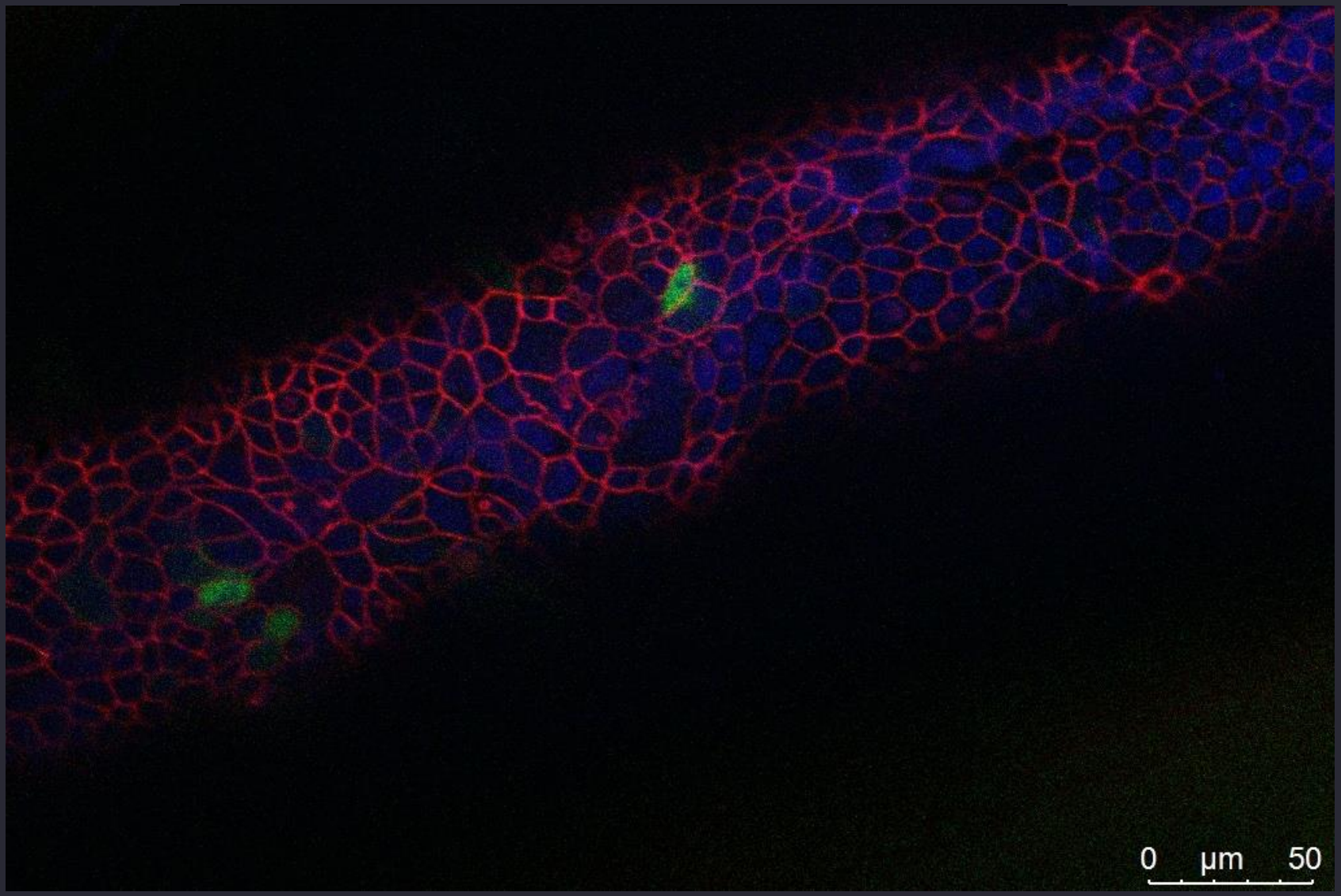
Toxicology in Vitro

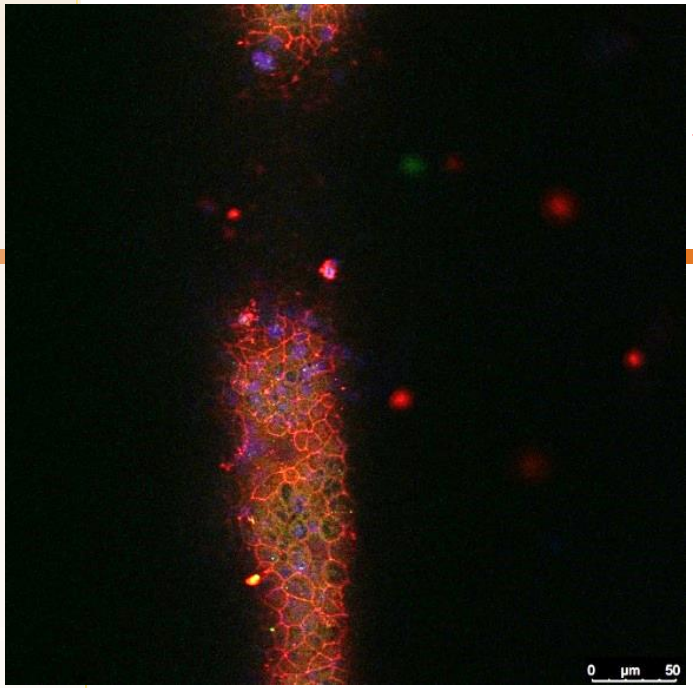
journal homepage: www.elsevier.com/locate/toxinvit

Development and validation of bioengineered intestinal tubules for translational research aimed at safety and efficacy testing of drugs and nutrients

Paulus G.M. Jochems[a], Jeroen van Bergenhenegouwen[a,b], Anne Metje van Genderen[a], Sophie T. Eis[a], Livia J.F. Wilod Versprille[a], Harry J. Wichers[c], Prescilla V. Jeurink[a,b], Johan Garssen[a,b], Rosalinde Masereeuw[a,*]

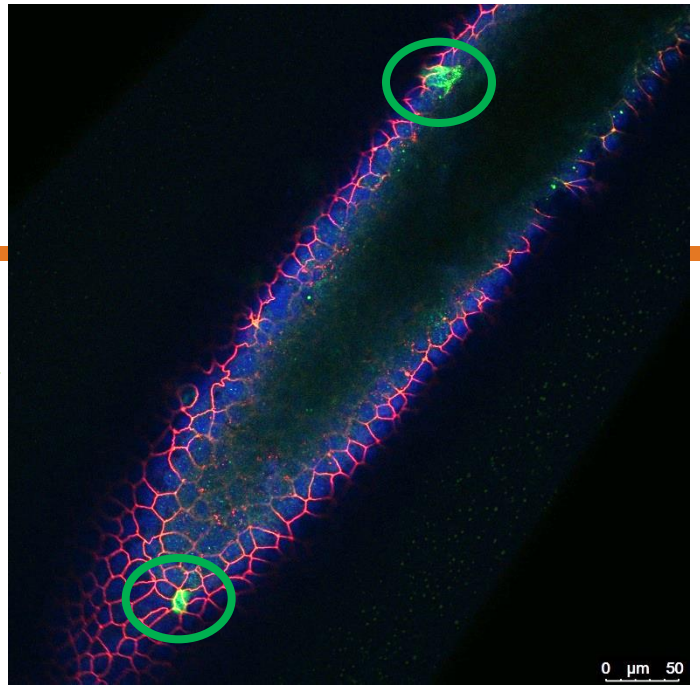
Bioengineered intestinal tubules





ZO-1 (red)
Tight junctions

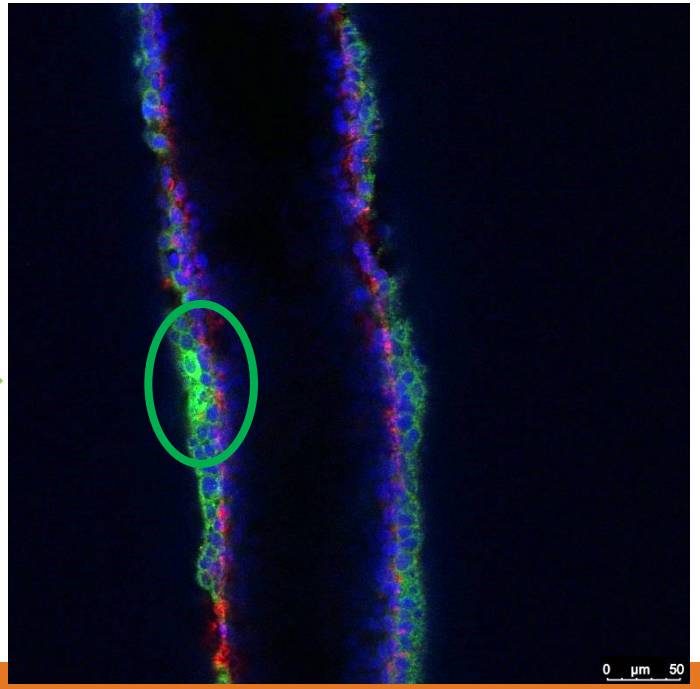
Mucin-2 (green)
Goblet cells



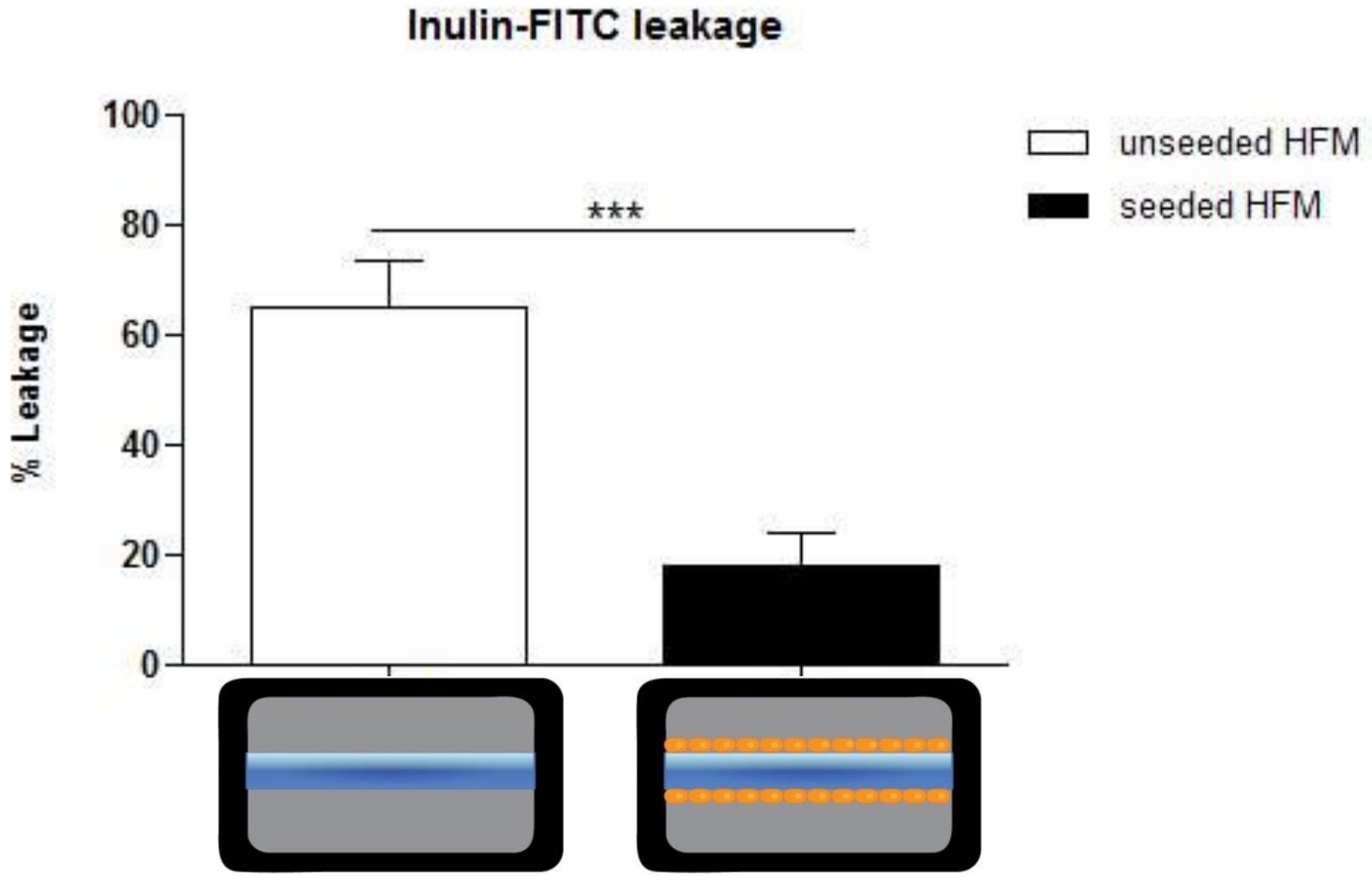
Differentiation

Lysozyme (red)
Paneth Cells

LGR-5 (green)
Stem cells



Bioengineered intestinal tubules



Conclusions

- Bioengineereed kidney proximal tubules **recapitulate** key epithelial features, suitable for **renal physiology**, **pharmacology** and **quantitative assessment** of tubular transport and mechanistic studies
- Similar approaches are used for **intestinal**, bile duct and liver tissue

Utrecht-Advanced *In Vitro* Models Hub

Utrecht-Advanced *In Vitro* Models Hub

- Many new, innovative *in vitro* models have significant potential to better predict human or animal physiology thereby replacing animal experimentation, but…
- development often stops after establishment due to:
  - lack of interest for implementation
  - lack of knowledge on validation
  - lack of funding

Utrecht-Advanced *In Vitro* Models Hub

- Aims to be a **leading centre of expertise** on development of *in vitro* models for diagnostics, models of disease, models for compound screening (chemical, pharmaceutical, food) and safety testing.
- Is a **one-stop shop** where high potential *in vitro* models are being developed, validated and transferred to industries and regulatory bodies.
- **Facilitates multidisciplinary collaborations** between academia, research institutes and industry, health care foundations and regulators.

Creating a center of expertise in Utrecht (U-AIM) for validation and valorization of advanced *in vitro* models with a strong focus on alternatives for animal experimentation is thus a timely investment.

Acknowledgements

UIPS Utrecht Institute for Pharmaceutical Sciences

Div. Pharmacology
Jitske Jansen
Manoe Janssen
Milos Mihajlovic
Michele Fedecostante
Katja Jansen
Paul Jochems
Carla Pou Casellas
Silvia Mihaila
Anne Metje van Genderen
Koen Westphal

UMC University Medical Center Utrecht

Nephrology & Hypertension
Marianne Verhaar
Karin Gerritsen
Maarten Rookmaaker

Biofabrication Utrecht
Jos Malda
Miguel Dias Castilho
Yang Li

Radboudumc

Dept. Pharmacology and Toxicology
Martijn Wilmer
Tom Nieskens
Janny Peters
Jelle Vriend
Frans Russel

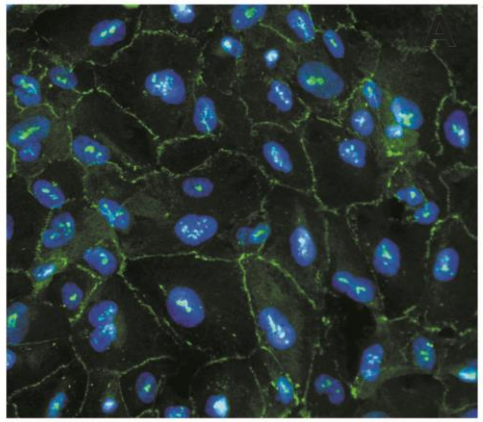
Dept. Physiology
Joost Hoenderop

Dept. Pediatrics
Bert van den Heuvel
Carolien Schophuizen

Dept. Nephrology
Luuk Hilbrands

UNIKLINIK RWTH AACHEN
Joachim Jankowski
Vera Jankowski

UNIVERSITY OF TWENTE.
Biomaterials Science and Technology (BST)
Dimitrios Stamatialis
Natalia Chevtchik

Universiteit Utrecht

Acknowledgements