Development of a humanized 3D kidney tissue model

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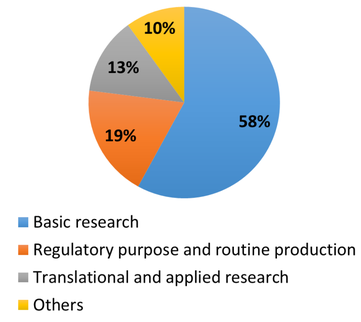
Introduction

1.9 million animals per year are used for animal experiments in Germany

70% Mice

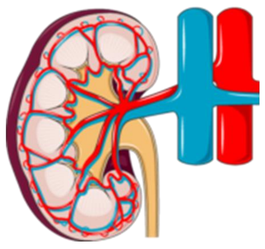
7% Rats

Animal experiments in Germany according to the purpose of the experiment (2020)



Aims and Objectives

The kidney is a very complex organ



- Up to 30 cell types
- Several functional units:
  - Glomerulus, proximal & distal tubuli, loop of Henle etc.

Problem

Available 3D in vitro models → only **one** functional unit

Glomerulus ⟷ (complex structure) Proximal Tubulus

Approach

Decellularization &

Recellularization with **human** cells

includes all units

humanized 3D-Kidney Tissue Models

- Basic research
- Toxicological testing
- Support future development

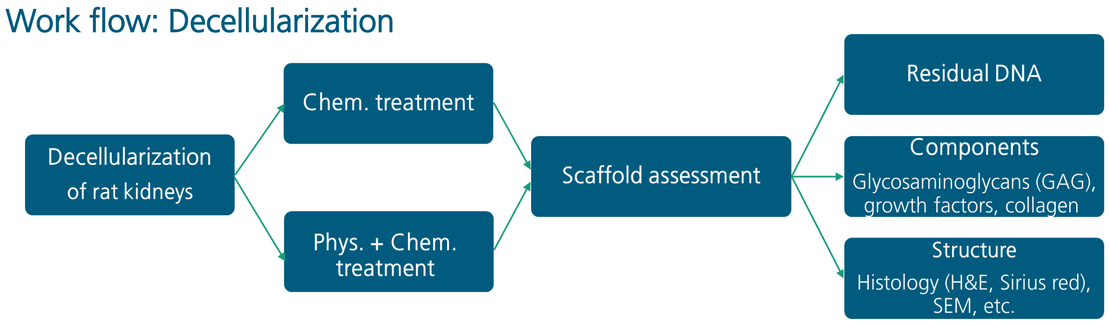
bioartificial kidney

- Chronic Kidney Disease (CKD) → >13% worldwide → ca. 2,4 Mio deaths/year
- CKD leads to end stage renal disease (ESRD)
- Treatment options: dialysis and transplantation

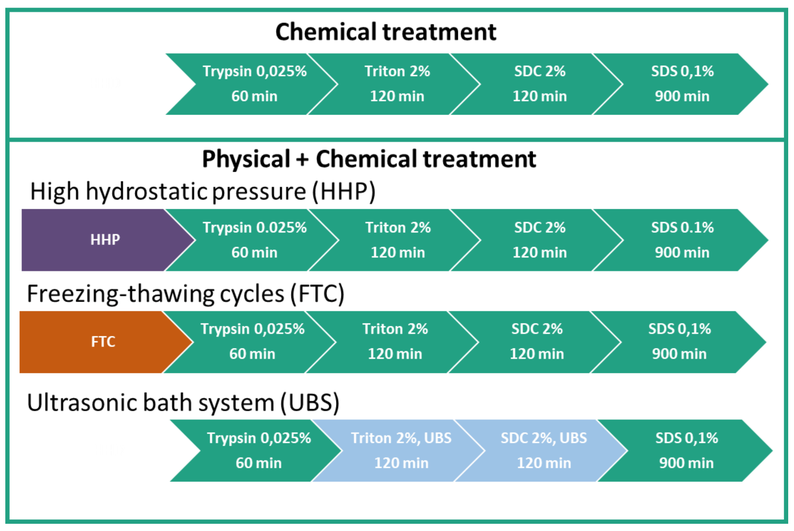
1909	7338
Kidneys were transplanted in Germany in 2020	Patients are waiting for a kidney transplant

Materials and Methods

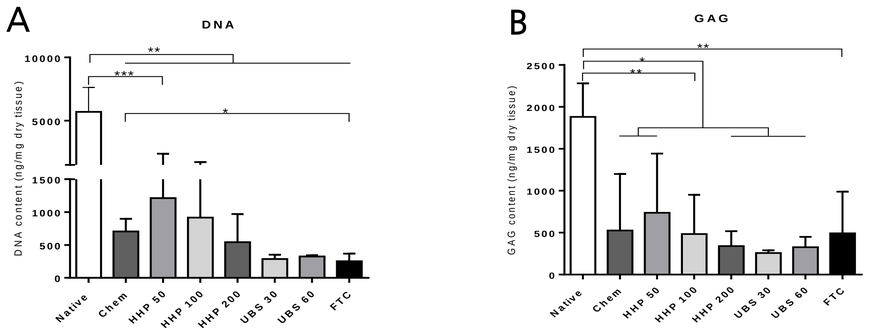
Work flow: Decellularization



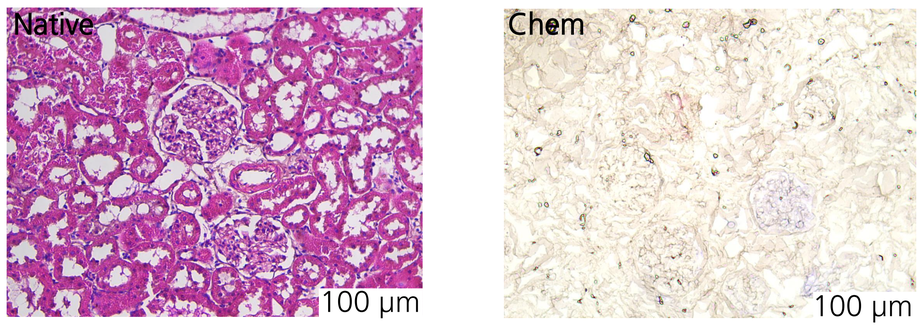
Decellularization protocols:



Results: Decellularization



*Amount of DNA and GAG in native kidney tissue and decellularized PCKS. While FTC resulted in a **significant** reduction in DNA content, its GAG content is partially preserved and comparable to Chem. n> 4.*



*Histology of **decellularized** PCKS stained with H&E (20x) shows removal of nuclei in Chem scaffolds. Chem resulted in an overall preservation of the structures with only minor damage*

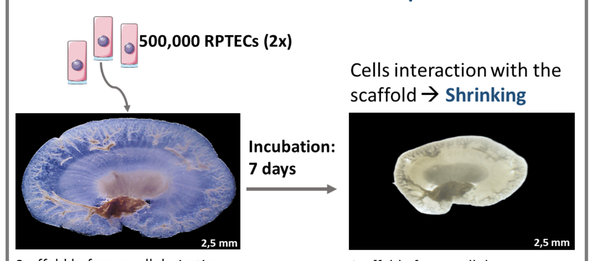
Scoring-Matrix: Converting descriptive microscopy data into quantitative data

Parameter	Chem	HHP 50 + Chem	HHP 100 + Chem	HHP 200 + Chem	UBS 30 + Chem	UBS 60 + Chem	FTC + Chem
Histology score	2.86	3.07	3.35	3.26	3.23	3.17	2.96
Composition score	2.9	2.9	2.4	2.7	3.1	3.0	3.3
Total score	* 2.87	3.00	2.88	* 2.96	3.17	* 3.08	* 3.10

* Selected for recellularization with human kidney cells (RPTEC: renal proximal tubular epithelial cells)

Results: Recellularization

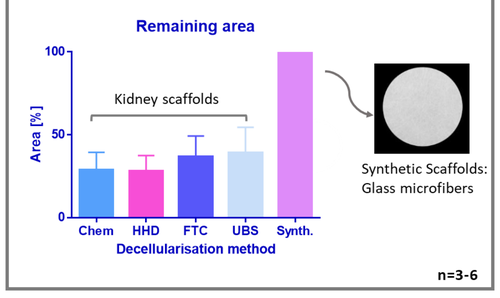
Static recellulaization → **method developed**

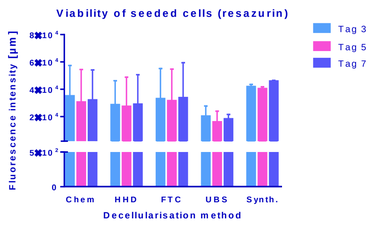


Scaffold before recellularization

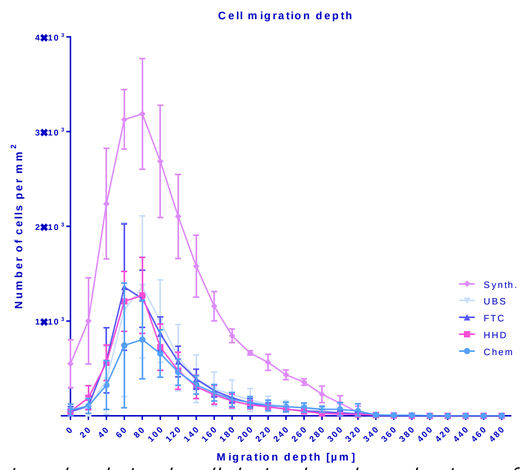
Scaffold after recellularization

Kidney scaffolds loose ca. **60-70%** of their area





Resazurin assay shows that seeded cells on UBS scaffolds have the lowest viability, while synthetic scaffolds have the highest viability. On the other hand, all other kidney scaffolds achieved a similar viability of seeded cells



Cell migration depth in decellularized and synthetic scaffolds. Seeded cells in all scaffolds (kidney and synthetic) reached a maximum depth of around 80 µm. Number of seeded cells in Chem scaffolds is less than other kidney scaffolds. Cell nuclei were stained with Hoechst 33342. Imaging was done with an epi-fluorescence microscope (WiScan, Hermes), n>4.

Summary

- FTC: highest reduction in residual DNA and a better preservation of GAG
- UBS: scaffolds have low ability for sustaining viable cells
- In process: investigation of specific attachment of RPTECs on their original positions

1 World Kidney Day, “Chronic Kidney Disease

2 New strategies in kidney regeneration and tissue engineering,” *Current Opinion in Nephrology and Hypertension*, vol. 23, no. 4, 2014.

3 Contribution of Physical Methods in Decellularization of Animal Tissues,” *Journal of medical signals and sensors*, vol. 11, no. 1, pp. 1–11, 2021.

4 I. Fischer, M. Westphal, B. Rossbach et al., “Comparative characterization of decellularized renal scaffolds for tissue engineering,” *Biomedical Materials*, vol. 12, no. 4, p. 45005, 2017.