Development of humanized 3D kidney tissue models from decellularized rat precision-cut kidney slices

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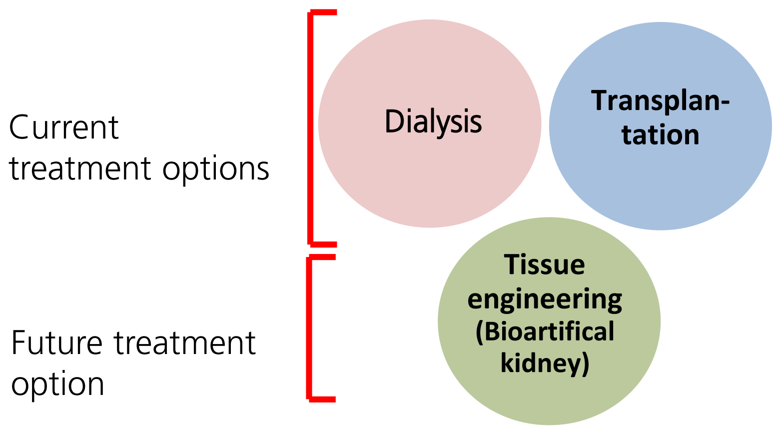
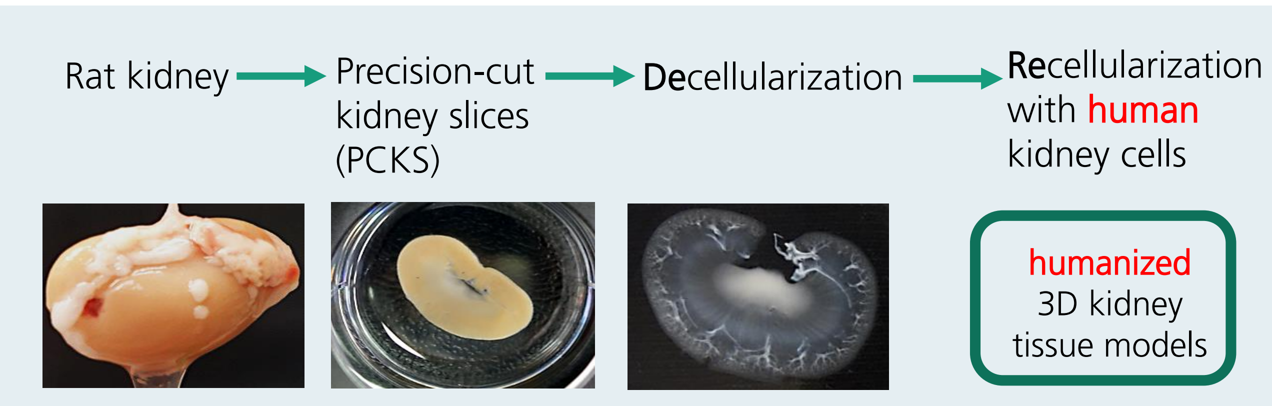
Introduction

Chronic kidney disease is progressing rapidly and affects more than **13%** of the world population [1,2].



Aims and Objectives

Aim: development of humanized 3D kidney tissue models



Why PCKS? De- and recellularization of **whole** kidneys is highly **complicated**

- PCKS better suited for the investigation of de- and recellularization strategies

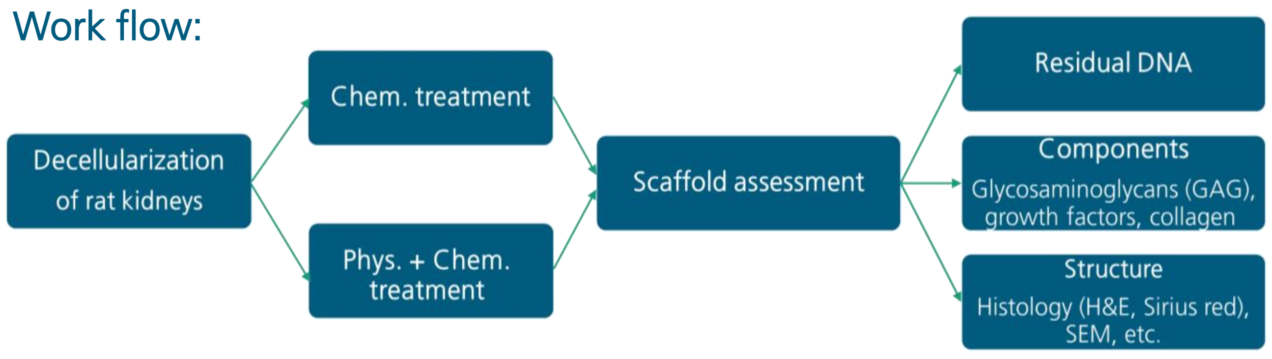
Benefit: **Reduce** the number of scarified **animals (12 PCKS/rat)**

Decellularization strategies: Pre-treatment with physical methods

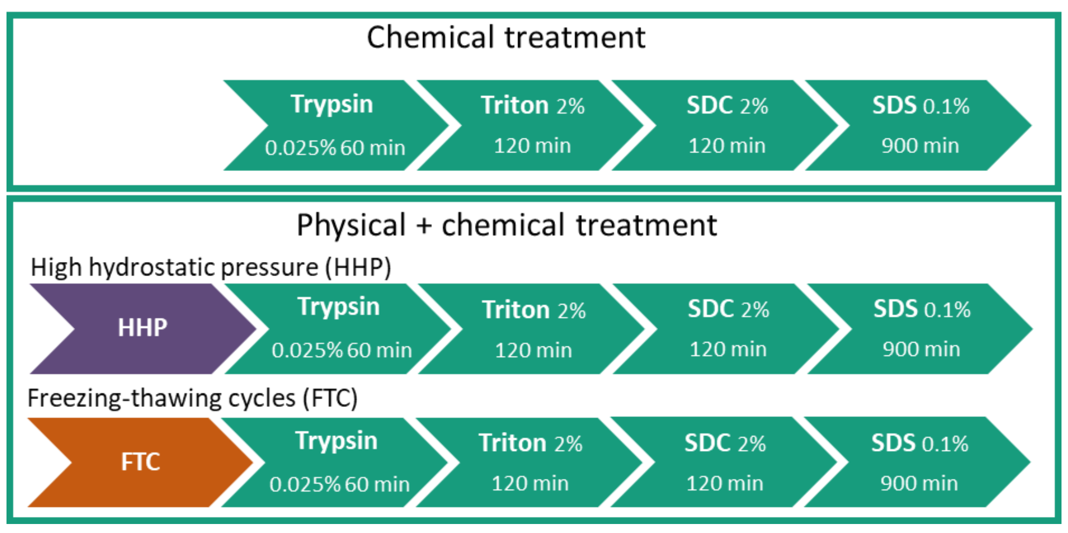
- Potential reduction in the duration of incubation in chemical reagents [3]
- Potential decrease of non-desirable damage of the extracellular matrix (ECM)

Materials and Methods

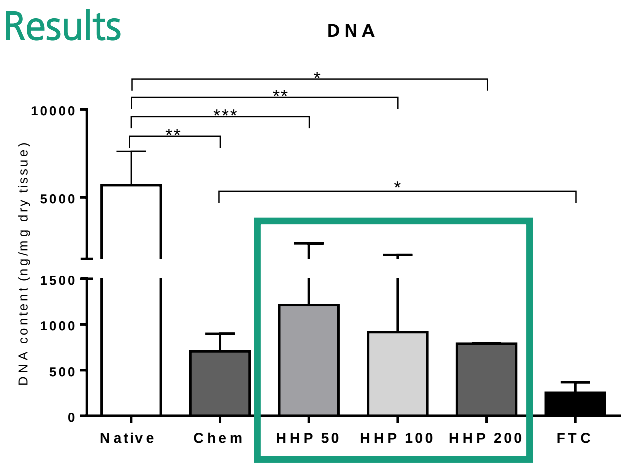
Work flow:



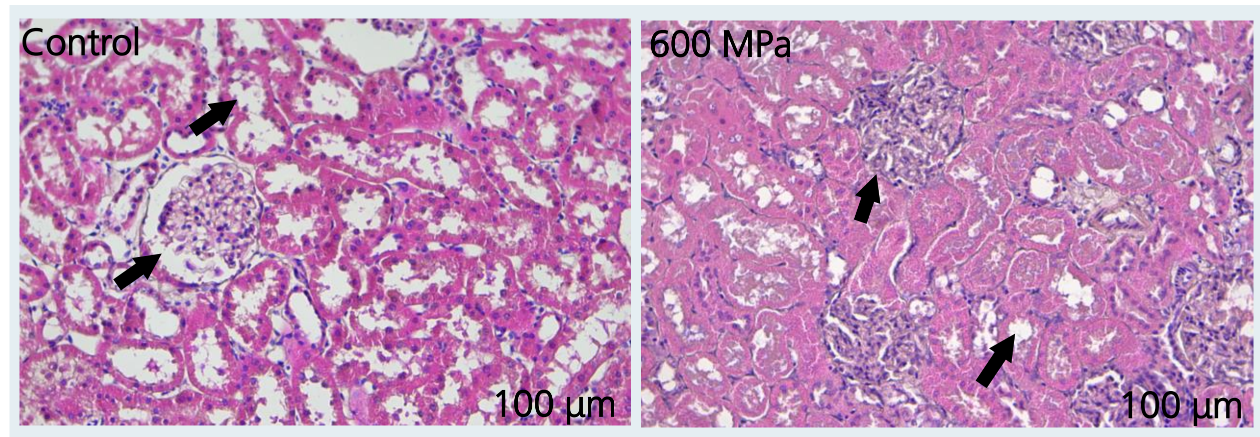
Decellularization protocols:



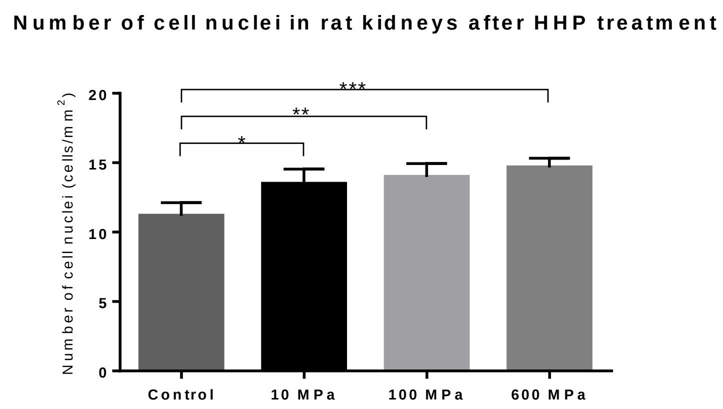
Results



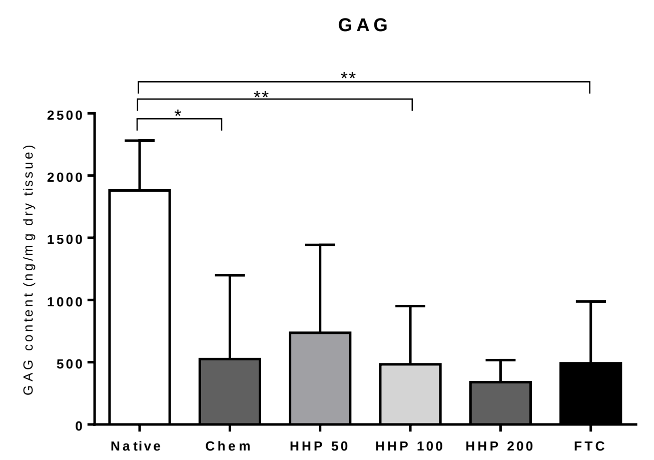
*Amount of DNA in native kidney tissue and decellularized PCKS. Chem (n=4), HHP 50 (n=5), HHP 100 (n=4), HHP 200 (n=2). While FTC resulted in a **significant** reduction in DNA content HHP protocols resulted in relatively similar DNA contents and non significant reduction compared to the Chem protocol. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001. Data were analyzed with a Mann-Whitney-two-sample-test and are given as mean (SD)*



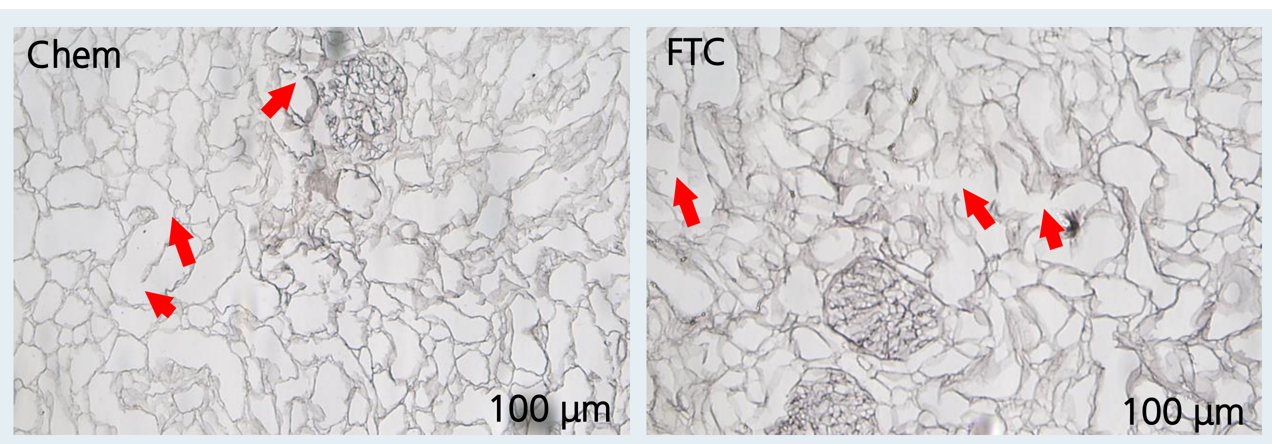
Histology of control and HHP (600 MPa) treated rat kidney tissues stained with H&E. The HHP treated tissue shows huge reduction in interstitial space (arrows) and a darker staining color



Number of cell nuclei in kidney tissues after HHP treatment. Control, 10 MPa, 100 MPa, 600 MPa (n=3). The data are normalized to the area of tissue of the control. Data are given as mean (SD). Cell nuclei were counted with QuPath and the tissue area was determined with ImageJ



*Amount of GAG in native kidney tissue and decellularized PCKS. Chem (n=4), HHP 50 (n=5), HHP 100 (n=4), HHP 200 (n=3). *P ≤ 0.05, **P ≤ 0.01. Data were analyzed with a Mann-Whitney-two-sample-test and are given as mean (SD). All Protocols resulted in a significant reduction in GAG amount compared to the native tissue. Chem and FTC protocols showed similar GAG content*



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

Summary

- FTC resulted in the highest reduction in residual DNA and a better preservation of GAG combined with only minor damage to the ECM
- HHP causes compression in kidney tissues leading to ineffective removal of residual DNA
- **In process**: further structural analysis with SEM
- **In process:** Recellularization of PCKS with renal proximal tubular epithelial cells (RPTEC/TERT1)

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.