High Hydrostatic Pressure for Tissue Devitalization and Development of Kidney 3D Tissue Models

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INTRODUCTION

The prevalence of chronic kidney disease (CKD) is progressing at a high rate affecting more than 10% of the world population. Current renal replacement options include either kidney **transplantation or dialysis**. However, dialysis does not replace all kidney functions. Hence, transplantation represents the preferred option in the case of end-stage renal failure, although life long immuno-suppression is needed. There is a high demand for donor organs and individuals in need of a kidney transplantation represent more than 80% of all patients on the waiting list.

Kidney tissue engineering

A third concept is under intensive research and development. Tissue engineering implies the removal of porcine kidney cells by **decellularization** and replacing them with patients own cells. Such bioengineered kidneys may possibly circumvent the respective limitations of the two methods currently used in the clinic.

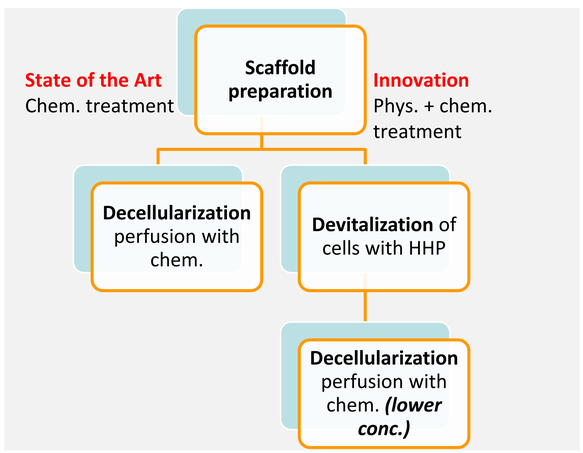
This project is part of the HOGEMA consortium, which uses **High Hydrostatic Pressure** (HHP) to effectively **devitalize** tissues without damaging the extracellular matrix (ECM).

AIMS AND OBJECTIVES

This project aims at applying HHP for the development of 3D kidney models:

- Investigation of the applicability of HHP to complex organs like the kidney.
- Investigation of recellularization strategies with the long-term purpose of producing **bioartificial kidneys**.

Work flow



MATERIALS AND METHODS

Kidney harvest

- Kidneys from male Sprague Dwaley rats (350-450 g) were used for decellularization and were cannulated *in situ.*

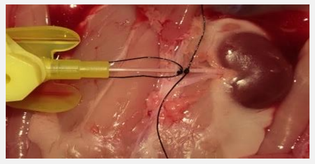


Figure 1: *in situ cannulated kidney*

Establishment of blood removal methods

- **TCP-Method:** kidneys were harvested from a **dead** rat and rinsed *in situ* by *transcardial perfusion* with heparin/PBS (5000 I.U/L).
- **Heparin-Method:** heparinization of a **living** rat (2000 I.U/Kg iv) and *in vitro* kidney perfusion with PBS.

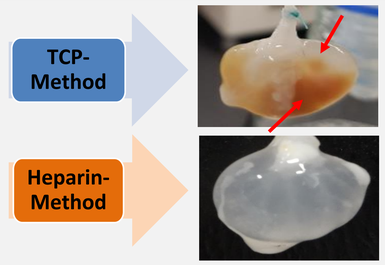


Figure 2: *Inhomogeneous decellularization of kidneys treated by the TCP-Method compared to homogenous decellularization of kidneys treated by the Heparin-Method*

Quantitative evaluation of blood removal methods

- Measurement of hemoglobin concentration in kidney tissues.

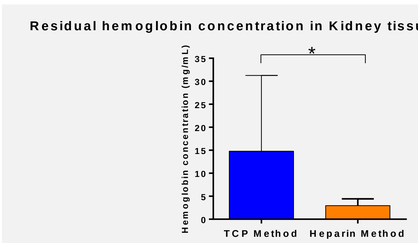


Figure 3: *Hemoglobin concentration in kidney tissue after blood removal with the "Heparin-Method„* ***(n=11)****, in comparison to the "TCP-Method„* ***(n=7)*** *P < 0,05. Data are given as mean (SD).*

RESULTS

Investigation of HHP effect on collagen denaturation

- **Objective:** best possible preservation of scaffold structure and components.
  - ECM consists of up to **90% collagen.**
- **Experiment:** tendons from rat tail were treated with HHP.
- **Assessment:** staining of rat tendon sections with the fluorescence-labeled "collagen hybridizing peptide" (F-CHP), which specifically binds to **denatured collagen**.
  - **No statistical significance** in fluorescence intensity between HHP-treated tendons (see figure 3 and 4).

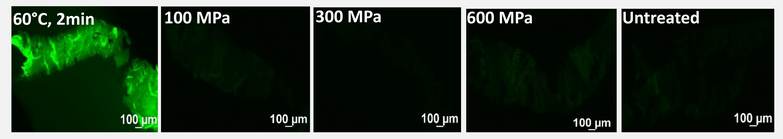


Figure 3: *Fluorescence imaging of F-CHP binding to denatured collagen in untreated and heat denatured tendon sections, as well as HHP-treated sections (100, 300 and 600 MPa)*

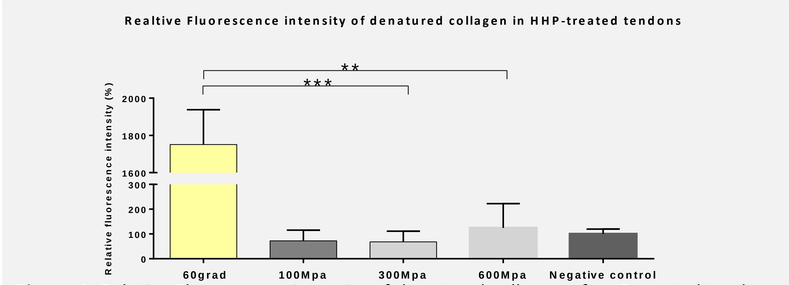


Figure 4: *Relative Fluorescence intensity of denatured collagen of HHP-treated tendons* ***(n=3, 2 different Rats)****, ***P ≤ 0.001, **P ≤ 0.01. Data are given as mean (SD).*

Exploration of HHP devitalization efficiency using precision-cut kidney slices

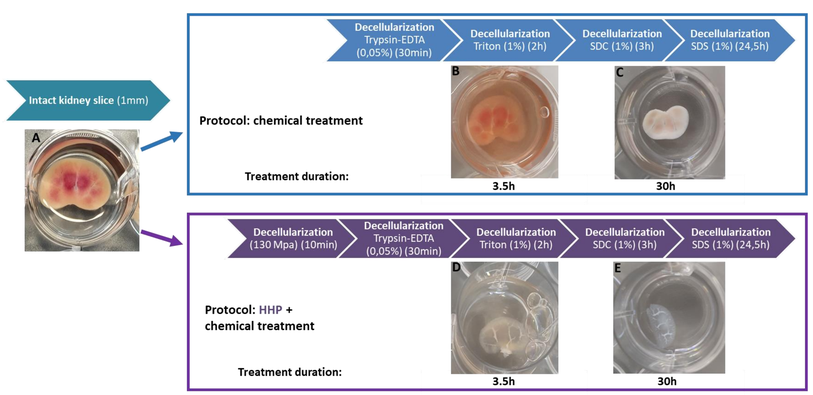


Figure 5: *The effect of HHP treatment on precision-cut kidney slices compared to chemical treatment alone and the corresponding decellularization protocols.*

A) Intact kidney slice, B) and C) Chemically treated kidney slices after 3.5h and 30h D) and E) HHP treated kidney slices after 3.5h and 30h.

Chemically treated slices are still not completely decellularized even after 30h of decellularization. On the other hand, HHP-treated slices are almost completely decellularized after ***only 3.5h.***

SDS: Sodium dodecyl sulfate, SDC: Sodium deoxycholate

CONCLUSIONS

- Treatment with HHP does not lead to collagen denaturation.
- HHP treatment can significantly shorten the detergents decellularization time and thus possibly reduce damage to the scaffold.

NEXT STEPS

A more precise assessment of the decellularization quality will be carried out in the upcoming phase of the project.

- Treatment of whole rat kidneys with HHP and further perfusion decellularization with chemicals.
- Assessment of decellularization efficiency and scaffolds quality:
  - **Structure:** histological staining (e.g. H&E), IF, IHC, TEM or SEM.
  - **Components:** quantification of Glycosaminoglycans (GAGs), growth factors and collagen content.
  - **Residual DNA**
- Scaffolds recellularization with 3-4 cell-types.

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