

High Hydrostatic Pressure for Tissue Devitalization and Development of 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 immunosuppression 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

In addition to dialysis and transplantation, a third concept is under intensive research and development. Whole kidney tissue engineering implies developing human kidneys from porcine kidneys by replacing the animal cells with human kidney 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 different tissues **without damaging** the extracellular matrix (ECM).

AIMS AND OBJECTIVES

- The applicability of HHP to complex organs like the kidney is central to this project with the goal to improve recellularization. Rat kidneys are used as a model for larger pig kidneys to analyze tissue development more effectively.
- Devitalization of rat kidneys by HHP followed by a washing process in comparison to chemical reagents based decellularization.
- Recellularization of kidney scaffolds with human cells such as iPSCs, endothelial cells, mesenchymal stem cells or human primary renal cells.

MATERIALS AND METHODS

Establishment of decellularization by perfusion

- Male Wistar rats (650-750 g) were used for kidney perfusion decellularization.
- Kidneys were washed out from blood according to the following alternatives:
 1. **Postmortem:** Kidneys were resected then rinsed *in vitro*.
 2. **Heparin Postmortem:** *in situ* rinsing by injecting heparin/PBS into the heart.
 3. **Heparin Premortem:** Subcutaneous heparinization (5000 I.U.) and *in vitro* rinsing.
- Kidneys were decellularized by arterial perfusion (SDS 0.1% at 2-10 ml/min).

RESULTS

Kidney Preparation and cannulation

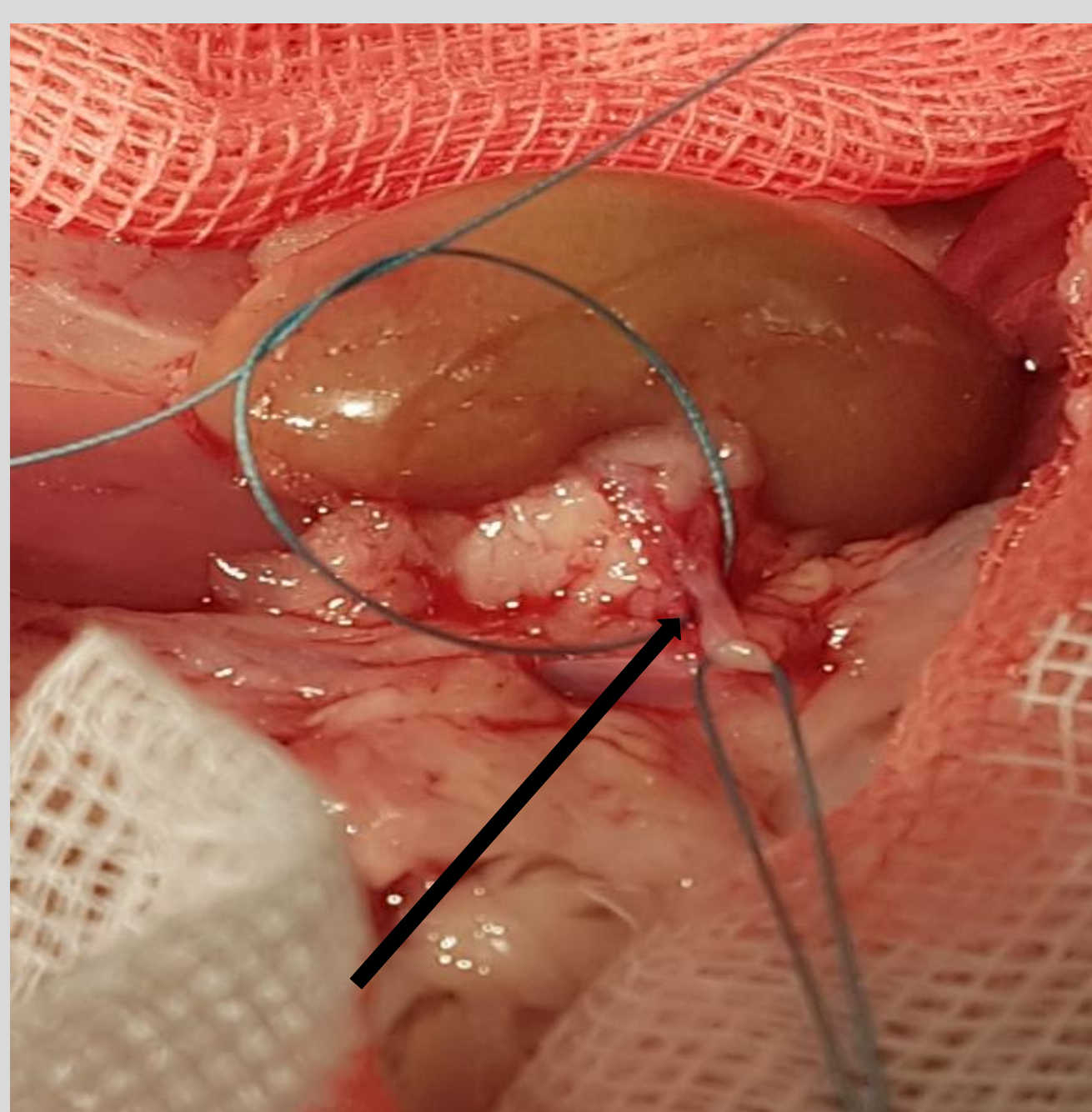


Fig. 1 In situ cannulation of the kidney artery. Arrow points towards on the artery

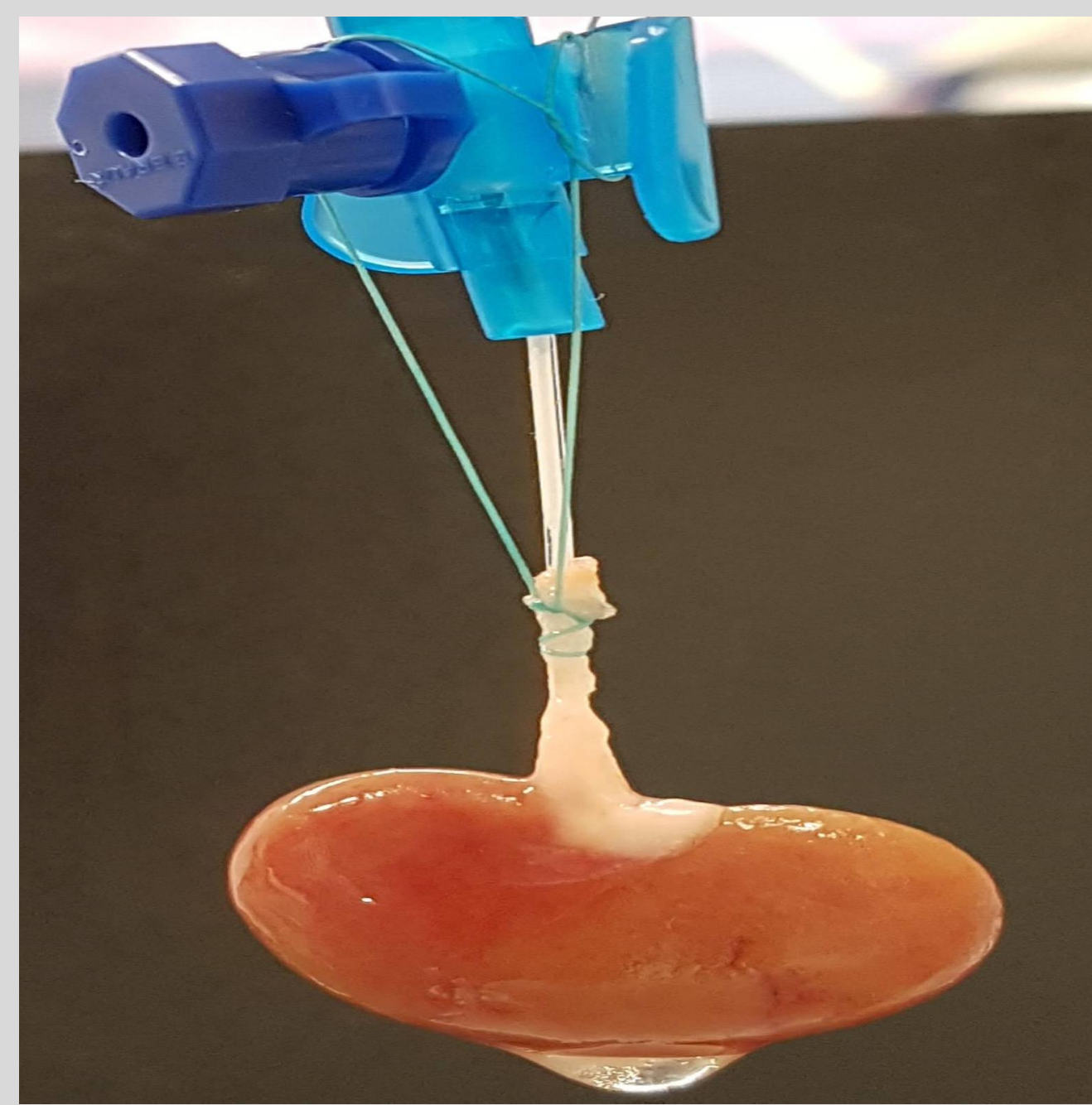


Fig. 2 In situ cannulated kidney

RESULTS

Decellularization by perfusion

1. Postmortem: in vitro renal blood rinsing



Fig. 3 Before blood rinsing

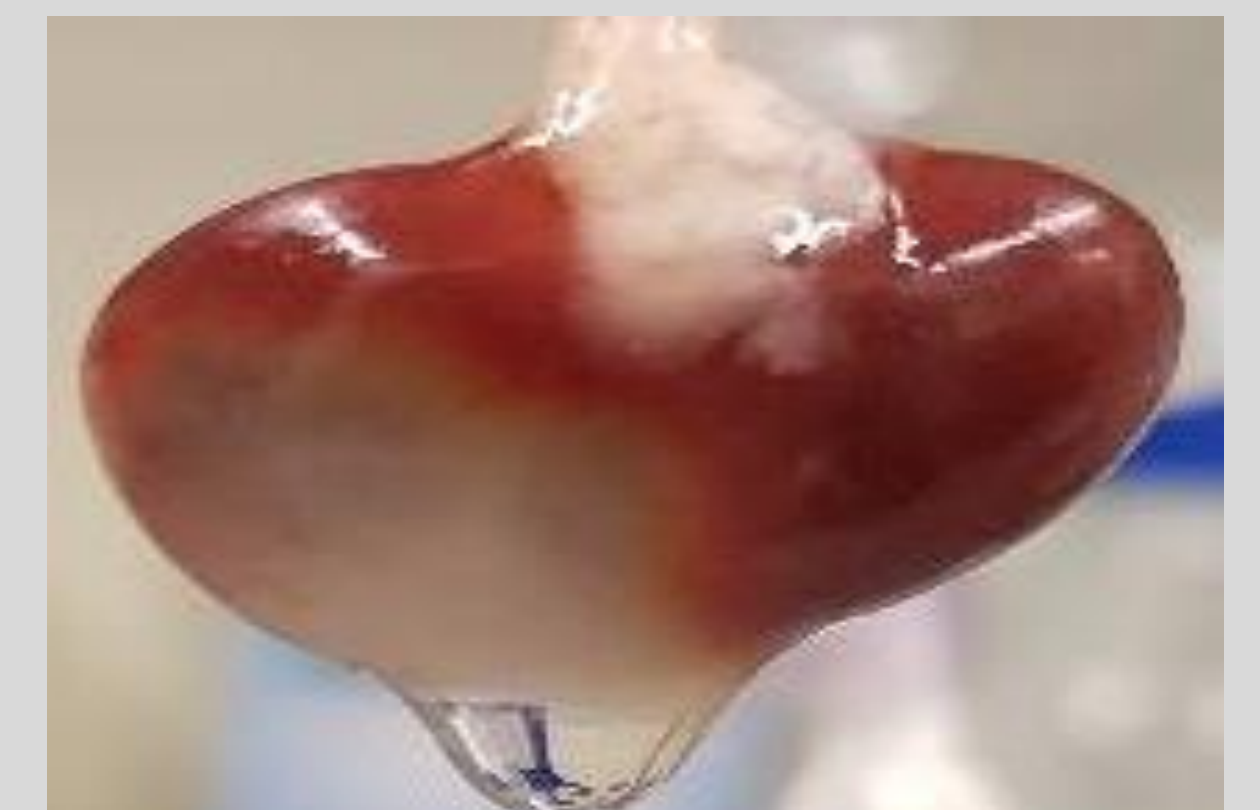


Fig. 4 Non-homogenous decellularization due to blood coagulation

2. Heparin Postmortem: in situ blood rinsing

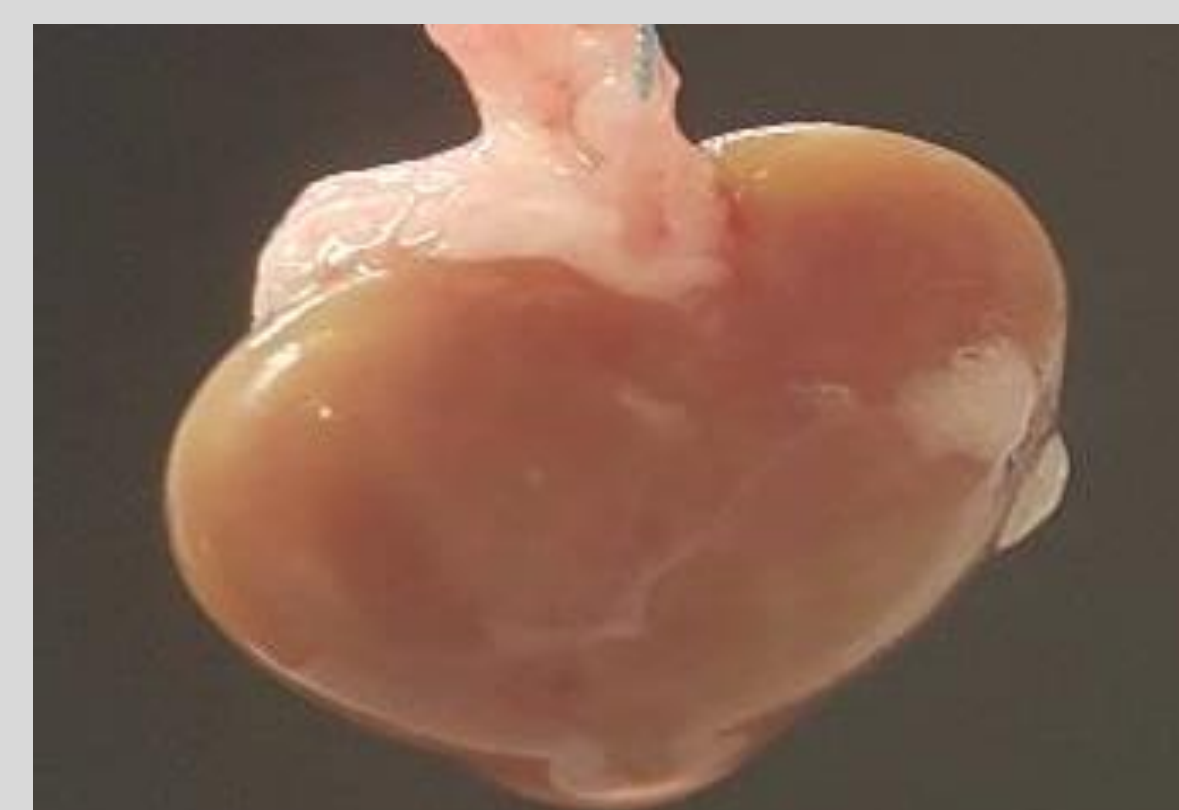


Fig. 5 After in-situ blood washing



Fig. 6 Blood coagulation prevented a homogenous decellularization

3. Heparin Premortem: Subcutaneous heparinization and in vitro blood rinsing



Fig. 7 Before blood rinsing



Fig. 8 homogeneously decellularized kidney

Hematoxylin and eosin (H&E) staining

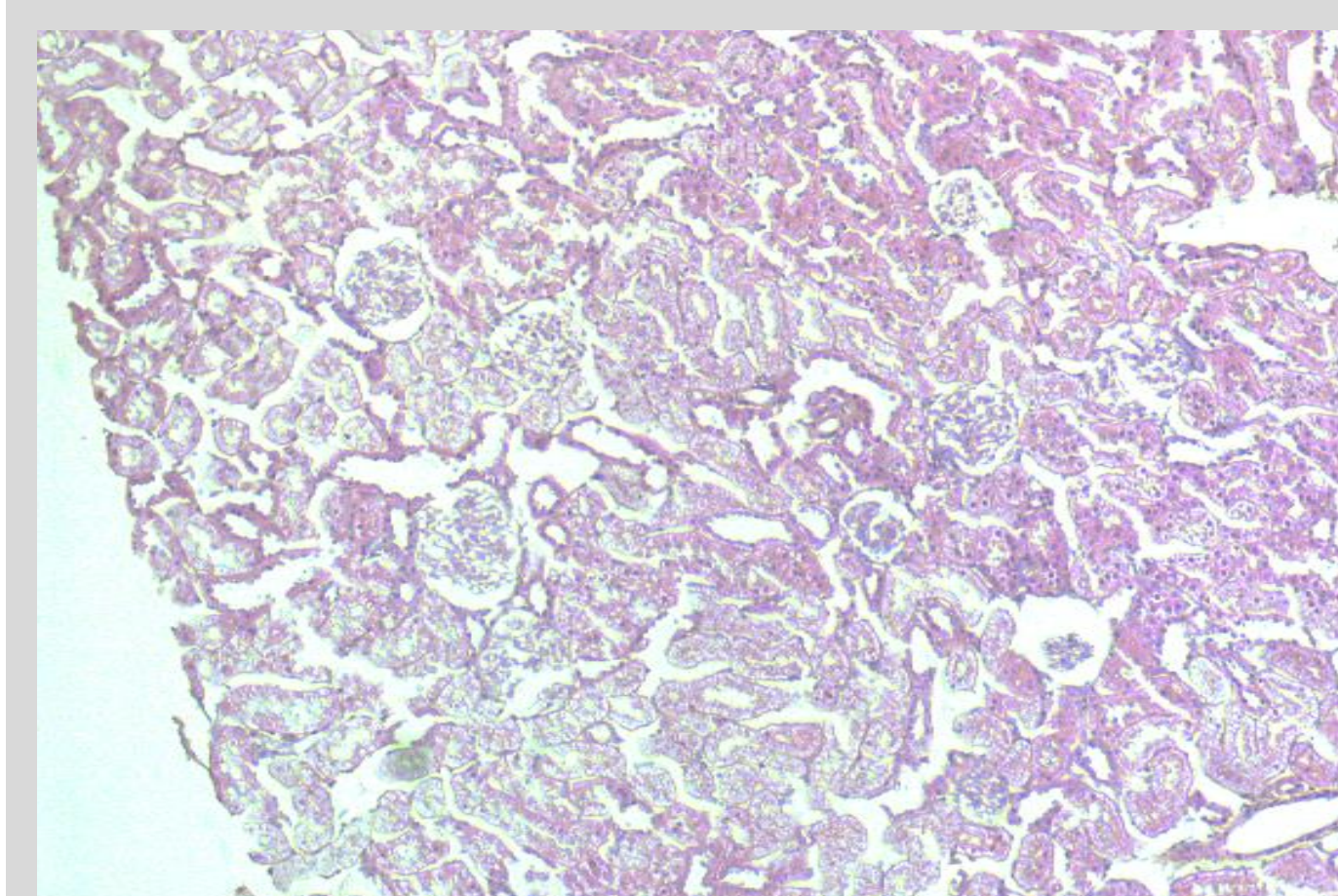


Fig. 8 Intact kidney

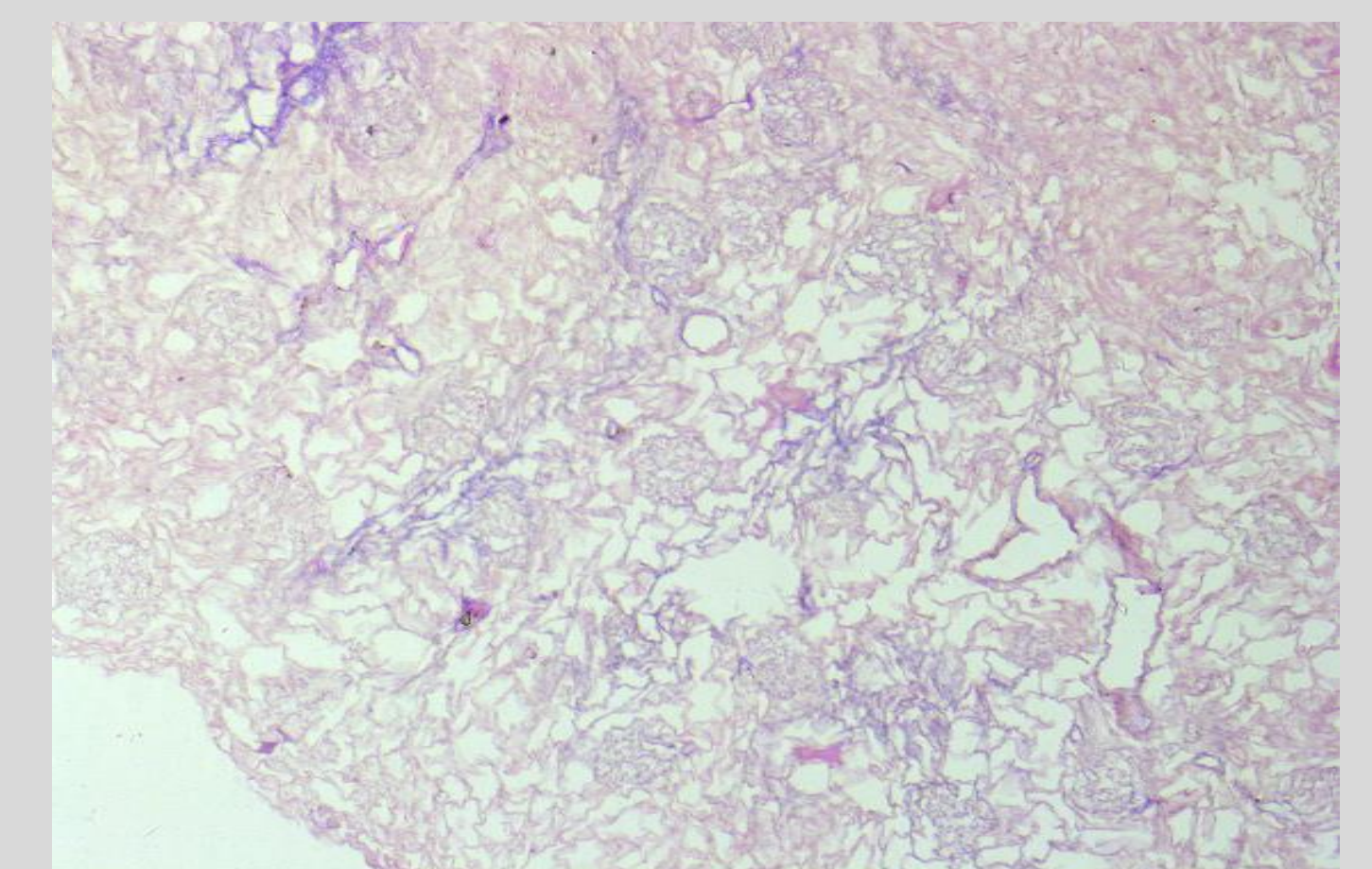


Fig. 9 Rat kidney decellularized by perfusion of SDS 0.1%

UPCOMING WORK

- Rat kidney devitalization by high hydrostatic pressure.
- Setting up a bioreactor for perfusion decellularization.
- Further analytical methods: immunofluorescence, live/dead staining, DNA quantification, ELISA, FACS.
- Rat kidney recellularization with human cells (e.g. iPSCs, endothelial cells, mesenchymal stem cells or human primary renal cells).