# Recellularization of decellularized porcine caval veins

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## Abstract

Decellularized material has been reported to be more suitable for cells to grow when compared to synthetic materials because repopulating cells are provided with the structural environment of native tissue. The aim of this study is to prepare and to test porcine caval veins through their decellularization followed by repopulation with human endothelial cells.

## Introduction

Although big steps have been done to provide a suitable material for vessels replacement in cardiovascular surgery, an optimal method is still lacking. Synthetic and autologous grafts have been already used when dealing with vessels failure.[1,2] Nevertheless, current research has focused rather on the replacement of arteries either with vein grafts or with arteries, with vein replacement being very rare. We hypothesize that the application of the vein graft into the same place where it was taken from could reduce the risk of graft failure derived from differences between veins and arteries due to blood flow related forces. There are only few articles that report the use of *in vitro* recellularized veins as vein graft in patients.[3,4] Another important issue is the availability of autologous vessels; it is not always possible to use a vessel from the same patient due to other health-related problems. On the other hand, when using a synthetic graft, there is a risk of infection and the need to use anticoagulants to avoid blood clots formation.[1] An acellular biological graft retains Extracellular Matrix (ECM) microstructure and cytokines supporting cell attachment and stimulation.[2] Once implanted in the patient, it does not require immunosuppression due to the removal of the original immunogenic components. Nevertheless, the risk of thrombosis is still high without the endothelial cells layer.[5] Thus it would be beneficial to reconstruct the vessel intima.[6] The aim of this project is to design and to construct a bioreactor for vessel re-endothelialisation, and to engineer vein grafts by seeding the endothelial cells in decellularized porcine caval veins.

## Materials and Methods

As vessels donors, Healthy Prestice black-pied pigs (ZD Mladotice, Czech Republic) aged 11–15 weeks and weighing between 30 and 40 kg are used.[7] Animals are premedicated by intramuscular administration of 10 mg/kg ketamine (Spofa, a.s., Czech Republic), 5 mg/kg azaperon (Janssen Pharmaceutica NV, Belgium), and 1 mg atropine (Hoechst Biotika, Slovak Republic). For general anesthesia are used propofol (1% mixture 5–10 mg/kg/h) (Fresenius Kabi Norges as, Norway) and fentanyl (1–2 µg/kg/h) (Chiesi cz s.r.o., Czech Republic). Animals are intubated and mechanically ventilated. After opening the abdominal cavity, the infrahepatic caval vein is exposed and all the lateral branches are ligated. Then the vein is extracted and stored in -80°C in saline with 10% DMSO until further processing. Animals are euthanized under anesthesia with a cardioplegic solution (KCl). All procedures are performed under approval of competent authorities and in compliance with EU legislation.

Vessels are decellularized using different cycles of 1% Triton X-100 and 1% Sodium Dodecyl Sulfate (SDS). To remove the detergents' residues from the scaffold, saline washes are performed. Hematoxylin and Eosin (H&E) staining, DAPI staining and residual DNA concentration are used to assess the decellularization results.[8]

*In vitro* repopulation with human umbilical vein endothelial cells (HUVECs) is done under both static and dynamic conditions. For the static seeding, veins are cut in small pieces and are then drop seeded with HUVECs. The culture is left in incubator at 37°C for 1, 3, 7 or 14 days. For dynamic perfusion the vessels are seeded with cells and then attached to a recirculating system in a bioreactor chamber.

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## Results

Firstly, vessel decellularization was optimized to obtain high quality vessel ECM scaffold. Complete cell removal was monitored microscopically by H&E staining and assessment of residual DNA (Figure 1a). Structure preservation of the scaffold was also confirmed. Repopulation of decellularized vessels was performed initially under static conditions, where cells were observed after 7 days incubation period. Further, a bioreactor was designed to seed and grow cells on the vessel in perfusion culture (Figure 1b). The growth medium flow rate was optimized as well as continuous medium oxygenation system. Dynamic

recellularization experiments are ongoing, with preliminary results from 3 days culture looking very promising.

## Discussion and Conclusions

We achieved successful vessel decellularization both in terms of time and scaffold preservation. Our preliminary results on static seeding and the development of a bioreactor for dynamic perfusion are the initial steps to obtain the final result. Studying decellularized grafts for vessel replacement is important to increase our knowledge and our ability to provide more suitable biomaterials for clinical use. If the characteristics of the native tissue are retained, the environment is attractive for cell attachment and growth as confirmed by our initial experiments with human EC. The implantation of a vein graft in the same position should also minimize the problems related to differences in blood pressure and bring in suitable mechanical properties. Further steps in this research are the *in vivo* proof of function of both decellularized scaffold and recellularized graft in a large animal model. This will allow us to better understand the scaffold behavior *in vivo* and can enhance the potential for clinical translation.

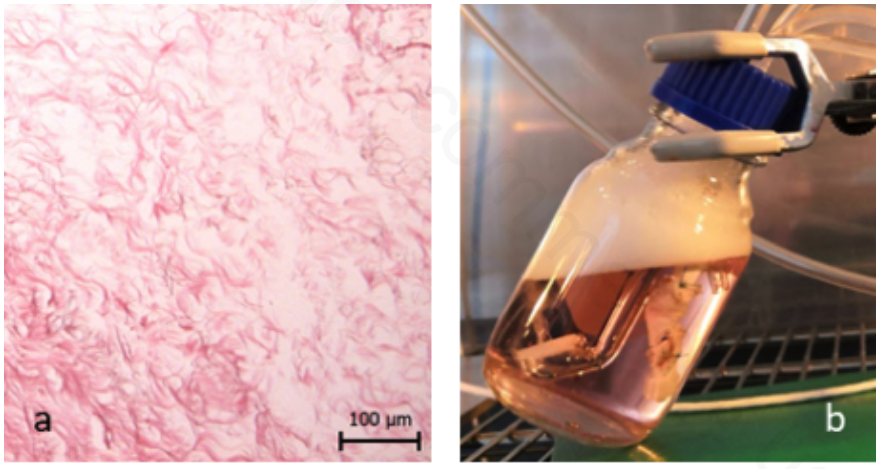


**Figure 1. a) H&E staining of the decellularized vein, b) bioreactor chamber for dynamic culture.**

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