ARE DECELLULARIZED PORCINE VESSELS SUITABLE GRAFTS FOR VASCULAR SURGERY?

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Introduction

Cardiovascular diseases are the leading cause of mortality worldwide, and the need for reliable solutions is still under investigation. Autologous vessels or synthetic grafts are commonly applied, but both present disadvantages [1]. Autologous vessels can cause comorbidities or are not available, while synthetic grafts have compliance mismatch and increased risks of thrombosis [2]. Biologically derived scaffolds are thus being studied for the presence of a natural environment capable of recellularization in vivo. Decellularization, the removal of cells, is leading the research to provide a suitable extracellular matrix (ECM) scaffold.
We investigated decellularized porcine carotid arteries and caval veins using mechanical testing, histological investigation and in vitro functionalization with human endothelial cells (ECs).

Methods

Both porcine carotid arteries and caval veins were explanted in our surgical facility in aseptic conditions with procedures approved by the ethical committee [3,4]. Vessels were cryopreserved at -80°C in a cryoprotectant (saline + 10% dimethylsulphoxyde). After thawing, perfusion of 1% Triton X-100 and 1% sodium dodecyl sulfate in 4 cycles of 1 hour each was applied. Hematoxylin-eosin (HE) staining was used to determine the efficacy of decellularization, Verhoeff's green trichrome, picrosirius red and Alcian blue with periodic acid Schiff were used to stain elastin, collagen and glycosaminoglycans respectively. Immunofluorescence (IF) stained fibronectin, laminin and vitronectin. Uniaxial tensile testing was applied on both native and decellularized carotid arteries to compare mechanical parameters. Seeding of ECs was performed both statically and dynamically in an in-house developed bioreactor.

Results

HE staining confirmed the absence of nuclei. Histological staining and IF evidenced the preservation of all the major ECM molecules. Mechanical parameters such as Young's moduli (elastic and viscoelastic), ultimate stress and ultimate strain varied only a little between the native vessel and the decellularized scaffold. This ECM proved to be suitable for ECs repopulation, with better outcome from the dynamic perfusion where cells were oriented regularly if compared to static seeding where cells were scattered and rounded (Figure 1).

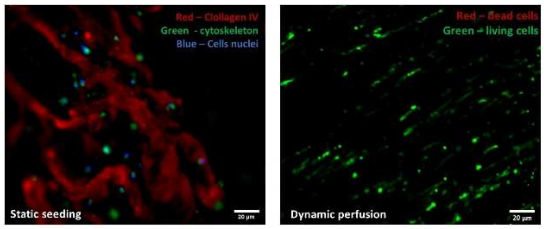

Figure 1: Repopulation of decellularized scaffold with human endothelial cells. Static seeding (left) was stained with immunofluorescence, while dynamic seeding was stained with a live/dead assay (right)

Discussion

The use of porcine vessels showed promising results for being used as vascular graft both in terms of structure and cells adhesion. The decellularization procedure had little to no influence on the mechanical properties and on the removal of ECM molecules responsible for both strength and cells adhesion. Perfusion of the seeded vessel in a bioreactor lead to a better functionalization of the lumen to limit platelet activation as a consequence of collagen exposure in vivo. Further investigations will involve the proof of function in vivo in a porcine model.

References

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