BIOLOGICAL VASCULARIZED MATRIX (BIOVAM) - SCAFFOLD DEVELOPMENT AND ASSESSMENT

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Introduction

Tissue engineering is a promising technique for reconstruction of failing organs. Based on its size the supply of cells with nutrients and oxygen in constructs requires an *in vitro* and/or *in vivo* vascularization e.g. a biological vascularized matrix (BioVaM). Here we report the generation of a decellularized matrix with a preserved vessel bed for the engineering of 3D artificial tissues. To generate artificial tissues for "proof of principle" experiments in a large animal model, the BioVaM was re-cellularized with porcine primary cells. The influence of residual surfactants on cell survival is discussed.

Methods

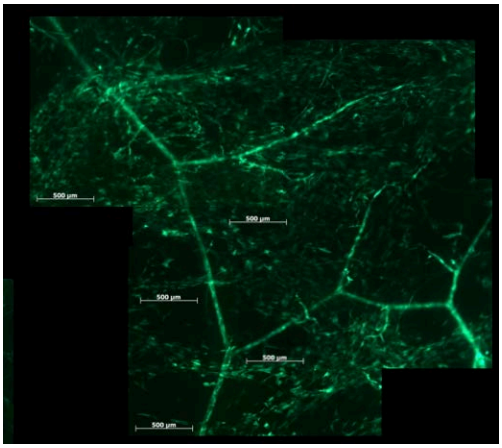
A decellularization process utilizing the surfactants Triton X-100 and sodium dodecyl sulfate (SDS) was used to generate a matrix with preserved pedicles derived from porcine small intestine. The decellularized matrix was intensively washed with PBS. The arterial and/or venous vessel bed was re-cellularized with lentiviral transfected primary porcine cells or a rat heart derived endothelial cell line (RHE). The re-seeded matrix was cultivated for 2 weeks under static and/or perfused conditions. Residual SDS was quantified in the supernatant of decellularized BioVaMs by a methylene blue assay. The critical concentration of SDS was determined for different cell types in cell culture experiments.

Results

Cell survival in the BioVaM was dependent on the cell type used. Complete re-cellularization was achieved with RHE and porcine smooth muscle cells. Primary porcine aortic endothelial cells perished after 6 days of cultivation. In the supernatants of BioVaMs, residual SDS was detected at a concentration of approximately 10 mg/l. Additional cell culture experiments revealed that the maximal SDS concentration tolerated by different cell types was 10 mg/l for primary porcine aortic endothelial cells and 50 mg/l for RHE cells.

Conclusion

SDS is broadly applied in decellularization protocols, but its high affinity to proteins makes efficient removal difficult. Although BioVaMs were intensively washed after decellularization, residual SDS was detected in the supernatant of the decellularized tissues. In cellular assays different cell types have a heterogenous tolerance to SDS, primary endothelial cells being the most sensitive. This is reflected in divergent re-cellularization efficiencies of the BioVaM depending on the cell type used. In summary, monitoring of SDS removal to generate high quality decellularized matrices is mandatory. Moreover, therapeutically relevant cell types should be applied for functional tissue re-cellularization rather than using robust cell lines often used in „proof of concept" studies.



pSMC after 7d culture in BioVaM