A Novel Whole Organ Bioscaffold with an Intact Vascular Network for Tissue Engineering and Regenerative Medicine Applications

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Statement of Purpose: A variety of naturally derived acellular scaffolds have been proposed for tissue engineering and regenerative medicine applications. However, their utility is limited by the lack of an intact vascular network. Preservation of functional vascular structures within the matrix system would allow for the enhanced engineering of three-dimensional tissues. In this study, we investigated the feasibility of developing a novel bioscaffold system with intact vascular network and tissue microarchitecture.

Methods: Cadaveric liver tissue was cannulated in the *vena cava* or portal vein and perfused using a decellularization solution in order to remove existing cellular components. DNA analysis, scanning electron microscopy (SEM) and histochemistry were performed to confirm the absence of cellular remnants and the preservation of structural integrity of the matrix. Fluorescence microscopy examination and fluoroscopic imaging were performed to demonstrate the maintenance and functionality of the vascular network. The scaffolds were seeded with various cell types, including vascular endothelial cells, primary hepatocytes and myoblasts, using a perfusion bioreactor system in order to assess the biocompatibility of the processed liver matrix. Bioscaffolds seeded with primary hepatocytes were implanted in athymic mice for cell survival and tissue formation *in vivo*.

Results/Discussion: DNA analysis and histological examination confirmed the absence of cellular components with the preservation of liver tissue structure and the vascular network. SEM imaging of the processed matrix further demonstrated the maintenance of liver tissue ultrastructure. Pentachrome and trichrome staining also indicated the preservation of elastin and collagen fibers. Perfusion with FITC-dextran and radiographic iodine contrast medium showed an impermeable portal-cava vascular system. The perfused endothelial cells attached and formed a monolayer on the luminal surface of the vascular channels. Primary myoblasts seeded on the matrix differentiated into skeletal muscle fibers within 1 week. Hepatocytes retained their phenotype and remained viable within the matrix during long-term culture. Hepatocyte-seeded implants demonstrated the presence of viable cells with adequate neovascularization by 1 week.

Conclusions: Our results demonstrate that a novel whole organ bioscaffold system can be developed using a perfusion decellularization process. We were able to preserve the native vascular network and tissue microarchitecture within the scaffold. Cells seeded on the liver matrix remain viable and progressively organize into tissue structures *in vitro* and *in vivo*. This technology may provide new opportunities in the engineering of vascularized three-dimensional tissue structures, critical for tissue engineering and regenerative medicine applications.