The Use of Whole Organ Decellularization for the Bioengineering of a Human Vascularized Liver

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Introduction

Our laboratory recently developed a decellularization method able to generate an entire organ scaffold from a whole liver, preserving its vascular network. Preliminary studies showed the possibility to efficiently re-cellularize the bioscaffold using perfusion cell seeding in a bioreactor. However, numerous technical issues remain to be addressed. The purpose of this study was to optimize the delivery of primary human liver progenitor cells in order to generate functional hepatic tissue.

Materials and Methods

Human endothelial cells (hECs) and freshly isolated human fetal liver progenitor cells (hFLPCs) were seeded through the portal vein of the bioscaffold. The seeded bioscaffolds remained in a bioreactor with constant culture medium perfusion up to one week. Microscopy was used to determine cell density and seeding efficiency. Immunohistochemistry was used to identify the engrafted cells and to detect hepatic tissue associated functions. Urea, albumin and prostacyclin secretion were quantified as parameters of cell function. Cell proliferation and apoptosis was also determined.

Results

The perfused hECs attached and formed a monolayer on the luminal side of the vascular channels. The primary hFLPCs showed heterogeneous seeding and high cell density in numerous large clusters distributed throughout the bioscaffold (with 1-1.5cm thickness). Immunohistochemistry showed progressive tissue formation with expression of albumin, alpha fetoprotein, cytochrome P450 3A, Hep-1 and EpCAM by the hFLPCs and von Willebrand Factor, VE-cadherin and eNOS by the hECs. Urea and albumin secretion was higher in the hepatoblasts seeded on the bioscaffold. Similarly, prostacyclin secretion was was higher in hECs seeded in the bioscaffold than hECs seeded in petri dishes. Widespread cell proliferation was also observed with Ki67 expression and modest apoptosis detected by TUNEL.

Discussion and Conclusions

Our results demonstrate the efficient generation of a bioengineered human liver organoid with hFLPCs and hECs using a perfusion cell seeding method in a liver bioscaffold. Hepatic and endothelial tissue functions and cell proliferation were detected with 3D tissue progressive formation in vitro. This technology may provide a new approach for liver bioengineering, critical for drug discovery and treatment of terminal liver diseases.