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Liver bioengineering: an emerging therapy for hepatic diseases

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Background: Tissue engineering holds a promise to transform transplantation medicine and drug testing. One of the most promising approaches to grow tissue in vitro is to seed decellularized tissue with autologous cells. Here we aimed to compare the two main methods to decellularize liver tissue and to reseed it with hepatocytes from animals of different ages.

Methods: To decellularize adult rat liver two protocols were used. The first method involved portal vein cannulation, followed perfusion with 1% SDS. The second method employed shaking individual hepatic lobes with 1% SDS and 1% Triton X-100 solution. After decellularization, perfusion with Phenol Red was used to confirm the integrity of hepatic vessels. As a cell source for recellularization primary rat hepatocytes were isolated from neonatal (1-2 day old), 6-week -old and adult (10-week-old) animals. CellTracker Red CMTPX fluorescent dye was used to monitor distribution of viable cells after recellularization. Metabolic activity of engrafted hepatocytes was examined using Bradford assay for albumin quantification. Finally, histological analysis of control, decellularized and recellularized samples was performed.

Results:Perfusion-based decellularization protocol successfully cleared liver from cellular content. Absence of cells in 0.1% SDS perfused liver samples was confirmed by Coomassie blue staining and histology. After five days of culture, hepatocytes from neonatal rats proliferated, migrated within scaffold material. In contrast, cells from 6 and 10-week-old animals exhibited lack of proliferation and poor adhesion to the decellularized liver scaffold. Hepatocytes from neonatal rats had significantly higher rates of albumin secretion as compared to their aged counterparts.

Conclusions: We have implemented liver decellularization and recellularization protocols using hepatocytes from rats of different ages. The data showed significant dependence between cell attachment and proliferation rates and age of donor animal.

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