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Production and characterization of acellular human liver matrix: potential auxiliary liver graft

Hakeem A, Wilshaw S P, Ingham E and Prasad K R
University of Leeds, UK

Shortage of donor organs combined with ever increasing liver disease has directed attention towards growing implantable liver tissue in the laboratory setting. Recent developments in decellularisation and recellularisation techniques have opened exciting facets for liver whole organ reengineering. The aim of this study was, to develop a decellularisation protocol for human liver, assess the structural and biochemical integrity and assess the biocompatibility of the acellular matrix. Discarded human livers were split and the left lateral lobes with a mean weight of 399 ± 162 g were used. Perfusion decellularisation was carried out through the hepatic artery and portal vein. Hypotonic buffer, 0.1% (w/v) sodium dodecyl sulphate, hypertonic buffer and nuclease solutions were used to remove cells plus nuclear material from the liver tissue. Following, several iterations of a previously patented Leeds University decellularisation protocol, greater than 91.0% DNA was removed from all areas of liver tissue. Histological analysis demonstrated lack of cells and maintenance of the portal triad histioarchitecture. Immunohistochemistry demonstrated positive staining for key extracellular proteins such as, collagen type I and III, fibronectin and laminin. Scanning electron microscopy showed an intact extracellular matrix. Biochemical assessment confirmed significant reduction in glycosaminoglycan content, but there was increase in collagen content, the latter likely due to extraction by dry weight of other soluble components in the matrix. The acellular tissues and extracts were not cytotoxic to either murine 3T3 or baby hamster kidney cells. Preliminary recellularisation work showed that the matrix is biocompatible to primary cryopreserved human hepatocytes seeded at 30,000 cells per m² density. The study has developed a suitable protocol for decellularisation of the left lateral lobe of human liver, without adversely affecting the extracellular matrix structure. The biocompatible acellular scaffold has the potential to be recellularised with hepatocytes, with a view to engineering a transplantable auxiliary liver graft.

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