Background

Alpha-1 antitrypsin deficiency (AATD) is a genetic disease caused by inactivation of the AAT gene. Accumulation of panacytolytic, mutated AAT in the extracellular matrix of afflicted hepatocytes leads to cell death which compromises the structural integrity of the liver parenchyma. AAT deficiency and its treatment cannot be efficiently repaired by the hepatocytes, there is a decade in circulatng AAT levels, which is in turn responsible for parenchymal enzymes and afflicated patients. The primary function of circulatng AAT is to protect normal liver tissues from damage by enzymes, such as pancreatic proteases, enzymes, microsomal and proteasome enzymes, for instance, resulting in fibrosis, cirrhosis and Kupffer cell activation, as well as exacerbating the liver injury suffered from AAT. Loss of the protective activity of AAT predisposes them to the development of lung damage and emphysema, while the accumulation of inosculating, panacytolytic mutant proteases in the CK of hepatocytes can result in significant liver injury. Whereas AAT replacement therapy is available for patients suffering from the pulmonary complications associated with AATD, the early definition therapy for the liver damage caused by AATD is liver transplantation.

Abstract

Conventional cell therapy and tissue engineering approaches to treating liver diseases and injury are limited by low cell retention, poor maturation, poor graft availability, and limited capacity for the restoration of normal liver function. Integration of next generation technologies such as 3D bioprinting is emerging as a key step towards the clinical success of these pioneering approaches and the potential for advanced approaches ranging from high-throughput systems of regeneration in ex-vivo liver failure to, we report fabrication, implantation and engineering of a human bioprinted therapeutic liver tissue (BTLT) containing human embryonic liver cells and liver endothelial cells (HLEC) and hepatocytes (PC) in a transgenic mouse model of alpha-1 antitrypsin deficiency (AATT). Following BTLT implantation in the surface of the liver in mice expressing the C-reactive protein (CRP) mouse, human hepatocytes were observed to secrete AAT at 56 days post-implantation. AAT levels were approximately 150 ng/ml at 125 days post-implantation. Histological evaluation of implanted BTLT revealed well-differentiated parallels of the tissue matrix with the underlying blood vessels with the implanted graft being defined along areas of parenchymal and non-parenchymal (NPCM) cells. The non-parenchymal areas contained perfused human endothelial vessels and non-parenchymal areas. Adjacent to the NPCM rich regions were areas of dense, packed cells, closely supported by cells phenotypically consistent with HLEC. The hepatocytes in the BTLT also displayed similar histological features, with critical transverse fractures. When compared to sham-operated,mock-implanted controls, BTLT implantation in the PO mouse resulted in an improvement of the pathological features associated with inflammation, including portal, within the hepatic lobules, stellate cells and Sirius red positive fibers were seen in the sham group. These results are indicative of an improved blood supply to the BTLT, successful engraftment of the liver with normal human hepatocytes and the vascularization of the liver. Reduced to no treatment options.

Biochemical Analysis

In vitro analysis of BTLT. ALP (c), revealing tyrosine activity within the parenchymal zone of the implanted tissue within 125 days of in vitro testing demonstrated enzyme activity of BTLT and donor populations of human hepatocytes within the BTLT. ALP-staining (red) and blue dyestuff (blue) staining (DAB) confirm the presence of mature human hepatocytes in the BTLT. The results reveal that the enzyme activity within the BTLT was significantly higher than sham operated controls at all time points.

Summary

- In vivo analysis of BTLT reveals in xenograft model tissues that contain extracellular matrix and receptor bioengineered, organized fibrous cells and hepatocytes that resemble native and metabolically functional. The BTLT developed mechanical/handling properties that allowed surgical implantation directly onto the liver of transgenic mice.
- Histological analysis of implanted tissues recovered after 90 and 125 days revealed large areas of globe donor hepatocytes adjacent to the implanted BTLT in treated but not sham animals, potentially preventing development of liver injury.
- Reduction in globule number of ~75% was seen in the treated vs. sham at 125 days post-implantation and reduced in the region surrounding the BTLT. A depth of ~400 microns.
- Human AAT detected in host livers for at least 125 days post-implantation could potentially be trypsin resistant to levels in the area, thus preventing the development of lung damage and emphysema that results from decreasing circulating AAT.
- Human AAT associated with liver function was detected in the host livers for at least 90 days post-implantation.
- The presence of native, perfused vascularization in the BTLT at 125 days post-implantation confirmed robust inflammation and cellular viability, a key limitation to traditional cell therapy approaches.

In summary, we have provided data supporting the regeneration, implantation, and engineering of a human liver tissue model for applications in a mouse model of human AATD. Our data supports fabrication of tissues that display key hepatic functions in vitro and the ability to confer functionality and efficacy upon xenograft regeneration, thereby providing an important tool to advance clinical development in translational therapeutics to confirm human protein production and graft function, with histological confirmation of graft benefit for 125 days. This approach to tissue fabrication shows potential to address liver issues related to cell replacement and disease that have limited the success of conventional cell therapy approaches to the treatment of liver diseases, because liver tissues are scalable and show therapeutic potential for patients currently have limited treatment options.