Long-term Performance of Implanted Bioprinted Human Liver Tissue in a Mouse Model of Human Alpha-1 Antitrypsin Deficiency

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Background

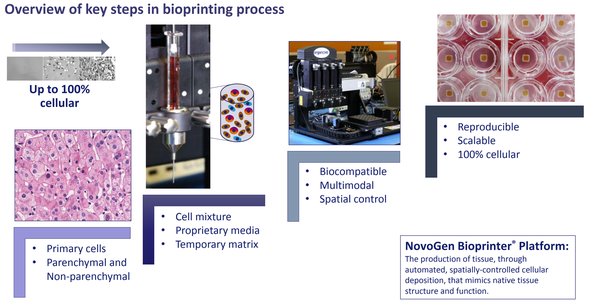
Alpha-1 antitrypsin deficiency (AATD) is a genetic disease, caused by mutation of the AAT gene. Accumulation of polymerized, mutated AAT protein in the endoplasmic reticulum of affected hepatocytes leads to cell death which in turn results in impaired liver function, fibrosis and, in some cases, hepatocellular carcinoma. Since the mutant AAT protein cannot be efficiently exported by the hepatocytes, there is a decline in circulating AAT levels, which is in turn responsible for pulmonary emphysema in afflicted patients. The primary function of circulating AAT is to protect normal body tissue from damage by nonspecific, neutrophil proteolytic enzymes, particularly neutrophil elastase, an enzyme that can attack lung elastin and compromise bronchial and alveolar wall integrity. For patients suffering from AATD, loss of the protective activity of AAT predisposes them to the development of lung damage and emphysema, while the accumulation of intracellular, polymerized mutant protein in the ER 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 only definitive 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 engraftment, poor graft durability and complications including portal hypertension. Integration of next generation technologies such as 3D bioprinting is an essential step towards the clinical success of these promising approaches and has the potential for broad applicability ranging from treatment of inborn errors of metabolism to acute on chronic liver failure. Here, we report fabrication, implantation and engraftment of a human bioprinted therapeutic liver tissue (BTLT) containing human umbilical vein and liver endothelial cells, hepatic stellate cells (HSC) and hepatocytes (Heps) in a transgenic mouse model of alpha-1 antitrypsin deficiency (AATD). Following BTLT implantation on the surface of the liver in mice expressing the mutant human form of alpha-1 antitrypsin (PiZ mouse), human albumin, transferrin, alpha-1 antitrypsin (AAT) and fibrinogen were detected in the circulation as early as 7 days, with increasing levels of human albumin detected for at least 90 days post-implantation. Histopathologic evaluation of implanted BTLT and underlying host tissue revealed integration of the fabricated tissues with the underlying host liver, with the implanted graft having defined areas of parenchymal and non-parenchymal (NPC) zones. The non-parenchymal zones contained perfused human CD31-lined vasculature and desmin-positive HSC. Adjacent to the NPC-rich regions were areas of dense, polarized Heps, closely supported by cells phenotypically consistent with HSC. The human hepatocytes in the BTLT also stained positive for albumin, AAT, fibrinogen and ornithine transcarbamylase. When compared to sham-operated, age-matched control animals, BTLT implantation in the PiZ mice resulted in an improvement of the pathological features associated with accumulated, misfolded protein within the mouse hepatocytes. There was an observed reduction in the accumulation of PAS-stained globule-containing hepatocytes adjacent to the implanted tissue. The reduction in hepatocytes containing large ER-bound globules was also confirmed by a decrease in ATZ11-positive cells in the host tissue. The rapid vascularization, durable tissue engraftment, target cell retention, and improvement in tissue pathology evince a promising novel approach to treating AATD and other liver diseases.

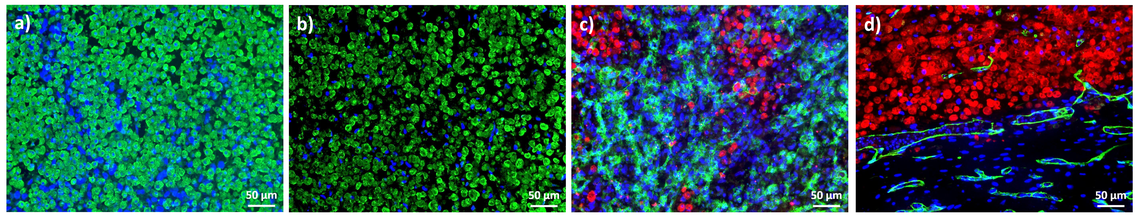
Key steps in generating Bioprinted Therapeutic Liver Tissue:

Overview of key steps in bioprinting process

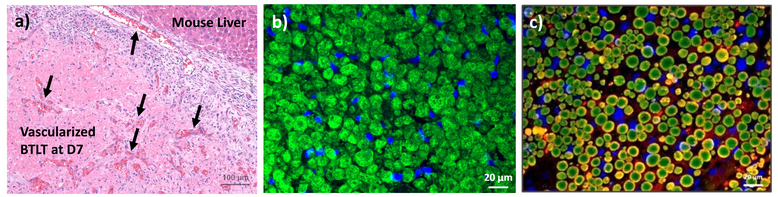


Overview of key steps in implanting BTLT

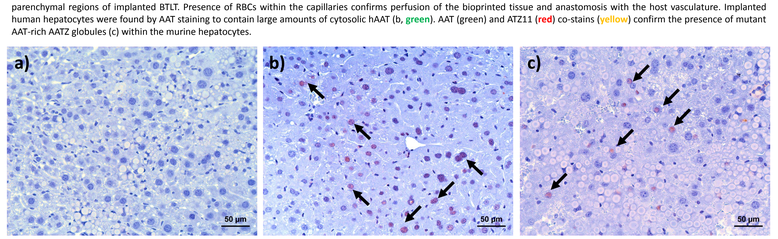
Histological Analysis



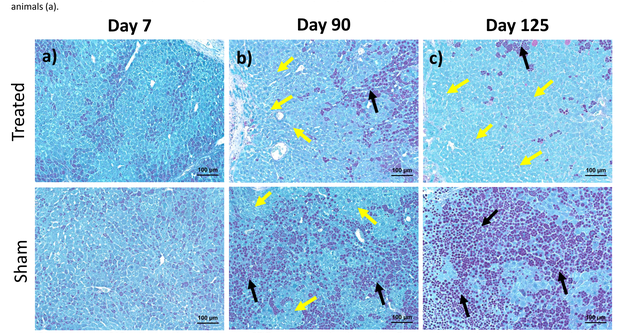
In vitro analysis of BTLT. hAAT (green) staining of primary hepatocytes within the parenchymal zone of the bioprinted tissue after 3 (a) or 21 days (b) of *in vitro* maintenance demonstrates cytosolic presence of hAAT and dense populations of human hepatocytes within the BTLT. BTLT stained for hAlbumin (red) and hCD31 (green) after 3 (c) or 21 (d) days reveal the presence of densely arranged human hepatocytes which are supported by organized networks of microvascular structures.



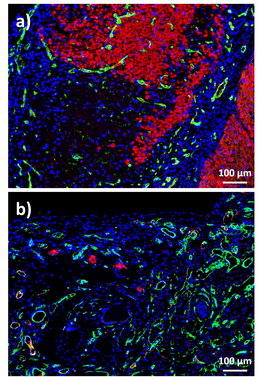
Assessment of developing BTLT after 7 days of implantation. H&E stained tissue sections (a) revealed abundant vascularization (black arrows) in both non-parenchymal and parenchymal regions of implanted BTLT. Presence of RBCs within the capillaries confirms perfusion of the bioprinted tissue and anastomosis with the host vasculature. Implanted human hepatocytes were found by AAT staining to contain large amounts of cytosolic hAAT (b, green). AAT (green) and ATZ11 (red) co-stains (yellow) confirm the presence of mutant AAT-rich AATZ globules (c) within the murine hepatocytes.



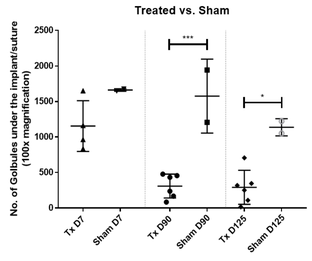
TUNEL-positive cells (black arrow) reveal apoptotic death of murine hepatocytes at D7 (b) and D125 (c) attributable to persistent ER stress in the sham-operated but not treated animals (a).



Diastase-treated PAS stained sections of implanted BTLT and surrounding tissue. After 7 days of implantation (a), there was no difference between treated and sham animals. The globules were small and uniformly distributed across the mouse liver. Following 90 days of implantation, areas of mouse parenchyma devoid of mutant AAT containing globules were seen adjacent to the BTLT (b, yellow arrows). After 125 days (c), globule-devoid regions were seen spanning larger areas adjacent to the implanted BTLT, while sham-operated control animals were observed to contain large areas of diastase resistant PAS-positive globules. Yellow arrows – globule devoid hepatocytes, Black arrows – globule containing hepatocytes.



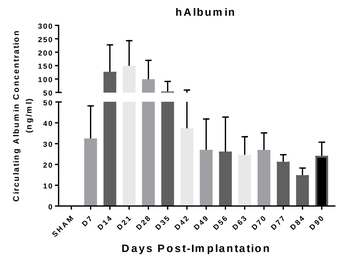
Immunofluorescent imaging of BTLT stained with hCD31 (green) at post-implantation Day 7 (a) or Day 90 (b) confirms the development of early stage microvessels at D7 (as also observed in a adjacent H&Es), which further mature and develop into larger caliber, human EC-lined vessels by D90. Robust halbumin (red) staining is seen in the tissues at D7 post-implant, identifying the implanted human hepatocytes.

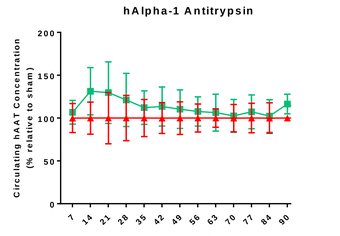


Ordinary one-way ANOVA of Treated vs. Sham
p value < 0.0001

Quantification of globules from the diastase-treated PAS-stained sections shows a significant decline in globule number in the treated mice as compared to the shams at D90 and D125 post-implant.

Biochemical Analysis





Bioprinted human liver tissue function. Whole blood was collected weekly from treated and control animals. ELISAs for hAlbumin and hAAT revealed the presence of human protein in mouse circulation for at least 90 days post-implantation, confirming successful engraftment of bioprinted tissues. hAlbumin was detected as early as 7 days in the treated animals, while there was no detectable hAlbumin in sham-operated controls. hAAT levels in treated animals (green) were higher than sham operated controls (red) at all time points.

Summary

- In-vitro maturation of BTLT results in compartmentalized tissues that contain endothelial cell networks, organized stellate cells and hepatocytes that maintain synthetic and metabolic function.
- BTLT developed mechanical/handling properties that allowed surgical implantation directly onto the liver of transgenic PiZ mice.
- Histological analysis of implanted tissues recovered after 90 and 125 days revealed large areas of globule devoid 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 treated vs. sham-operated animals at 125 days post implantation, in the region surrounding the BTLT, to a depth of approx. 1mm.
- Human AAT detected in host circulation for at least 90 days post-implantation could potentially help supplement AAT levels in the sera, thus preventing the development of lung damage and emphysema that results from decreased circulating AAT.
- Human albumin associated with liver function was detected in the host circulation for at least 90 days post-implantation.
- The presence of mature, perfused vasculature in the BTLT at 90 days post-implantation confirms robust engraftment and cellular retention, a key limitation to most traditional cell therapy approaches.

In summary, we have presented data supporting the generation, implantation, and engraftment of bioprinted therapeutic liver tissue 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 implantation. Serum analysis was conducted for 90 days to confirm human protein production and graft function, with histopathologic confirmation of graft benefit for 125 days. This approach to tissue fabrication shows potential to address key issues related to low cell engraftment and dosing that have limited the success of conventional cell therapy approaches to the treatment of liver diseases. Bioprinted liver tissues are scalable and show therapeutic potential for patients that currently have limited to no treatment options.