Bioprinted three-dimensional (3D) human liver constructs provide a model for interrogating liver biology
Justin B. Robbins, Colin M. O’Neill, Vivian Gorgen, Sharon C. Presnell, and Benjamin R. Shepherd
Tissue Applications and Systems Engineering Groups
Organovo, Inc., 6275 Nancy Ridge Dr., Suite 110, San Diego, CA, USA

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Abstract
Conventional two-dimensional (2D) cell culture models do not reflect the complexity of biological phenomena that occur in the liver microenvironment, leading to mispredictions in drug development and toxicity models. One of the major reasons for these differences is that primary hepatocytes, when isolated and cultured in a 2D monolayer, rapidly lose key regulatory properties that are consistent essential for prediction modeling. Loss of these key phenotypic features is one of the major limitations in toxicology screening protocols involving human hepatocytes, which have shown the pace of development for new drug entities. In addition to challenges in maintaining differentiation, species-specific differences in hepatocellular function have also been extensively reported, making it difficult to extrapolate non-human data to the clinic. Therefore, providing better in vitro models for interrogating human liver biology will have a major impact in the fields of toxicology and hepatology. In this report, we demonstrate that 3D bioprinted liver tissue constructs composed of parenchymal human primary hepatocytes and hepatic cell line (L02) and non-parenchymal cell populations (stellate cells (HSC)) are capable of supporting human liver function for 14 days and maintain key biochemistry and function. The 3D liver tissues are metabolically active for at least 42 days, producing essential liver-derived serum proteins including albumin, fibrinogen, and transferrin within the medium. Histological analysis of formalin-fixed paraffin-embedded tissues for the expression of liver-specific markers demonstrates tissue-like hepatocyte morphology and function over extended time periods. Bioprinted tissues demonstrated tissue-like performance over extended time periods and maintained key liver phenotypic features over time. In conclusion, bioprinted liver tissues exhibit several key attributes that are critical for the development of new drug entities.

Conclusions
Organovo’s unique bioprinted 3D liver tissues exhibit several key attributes including:
- Cellular composition closely mimicking human liver tissue
- Development of well-organized, tissue-like architecture ~250 µm thickness
- Sustained metabolic activities over time
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These data further support the use of 3D bioprinted liver tissues as a novel system to interrogate liver biology both biochemically and histologically.

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Figure 3. 3D bioprinted tissues show tissue-like performance over extended time periods. A.) Bioprinted tissues exhibit dense tissue-like architecture demonstrated by H&E staining at day 7 (**). B.) Performance of 3D liver tissues over 42 days was validated by measuring albumin production over time.

Figure 4. 3D bioprinted tissues possess liver-associated features and function for extended time periods. Bioprinted tissues demonstrated tissue-like light junctions as measured by E-cadherin (A.) and Claudin (B.) stains at day 3. C.) Performance of 3D liver tissues over 14 days was validated by measuring albumin production over time. D.) Liver tissues treated with diclofenac after incubating for 14 days in culture, demonstrate the expected induction of CYP3A4 at moderate concentrations, while at high concentrations, damage is apparent by lactate dehydrogenase (LDH) induction and concomitant decrease in CYP3A4.

Results

Media Control Vehicle Control APAP (75 mg/ml)

LDH Activity APAP

Figure 5. Bioprinted 3D liver tissues can effectively model hepatotoxic insults. A.) Viability of liver tissue is diminished by treatment with acetaminophen (APAP) +/- ethanol (EIOH), as shown by a dose dependent decrease in ATP (Cell Titer Glo; Promega). Addition of EIOH shifts the LD50 APAP ~2 fold. B.) Increases in LDH indicate tissue damage in the presence of increasing doses of APAP. H&E staining of liver tissues (non-treated controls (C. and D.)) shows pronounced necrosis in those treated with APAP (E.).

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