A novel *in vitro* three-dimensional bioprinted liver tissue system for drug development

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Abstract

Despite efforts to improve the ability to identify the toxicity of therapeutic compounds, the attrition rate for both experimental and approved drugs remains very high. Cardio- and hepatotoxicity remain primary reasons for late stage failures and post-market withdrawals. Therefore more robust human, *in vitro* models of these organ systems are needed. We have developed a bioprinted th-ree-dimensional (3D) liver system that captures several key features of in vivo tissue, in a multi-well format suitable for drug screening. Using NovoGen™ bioprinting technology we have fabricated 3D liver constructs containing architecturally- and physiologically-relevant features for two hepatic cell lines and primary hepatocytes, within standard multi-well culture plates. Bioprinted, 3D hepatic neotissues were further enhanced in complexity with the addition of endothelial and hepatic stellate cells. Biochemical studies demonstrate that several critical liver functions are present including cytochrome P450 activity. Tight junction protein expression was observed throughout the 3D tissue. Analysis of cell death and proliferation following in vitro maturation revealed the constructs were viable. These results demonstrate a flexible bioprinting method to rapidly fabricate multi-cellular 3D liver constructs in a multi-well format enabling both drug screening and interrogation of liver biology.

Materials and Methods

Cell culture. All cells utilized in these experiments were sourced from commercial vendors and cultured according to the manufacturer's recommended protocols. Cryo-preserved primary hepatocytes were purified by Percoll gradient centrifugation prior to use in bioprinted constructs. iPSC-derived hepatocyte-like cells (iCell Hepatocytes) were generously provided by Cellular Dynamics, Inc.

Bio-ink preparation. Bio-ink was prepared based on protocols and techniques described in Norotte et. al., (Biomaterials 30: 5910-5917). For preparation of hydrogels containing cells, NovoGel 2.0 (Organovo, Inc.) was prepared according to manufacturer's instructions and non-parenchymal cell populations were incorporated prior to bioprinting.

Bioprinting. All tissues were fabricated directly into standard tissue culture plates (Corning Transwell) using standard Organovo bioprinting protocols and the NovoGen MMX Bioprinter or modifications thereof.

Secreted protein detection. Spent media was analyzed by commercially available ELISA kits for the following liver metabolites: albumin, fibrinogen, and transferrin. Cholesterol biosynthesis was quantified fluorimetrically (Cayman Biochemical).

CYP P450 analysis. CYP1A2 and CYP3A4 activity was assessed with the Pro-Glo™CYP P450 Assay system (Promega). Liver tissues were challenged with either verapamil (10μM) or dexamethasone (10μM) to stimulate CYP1A2 or CYP3A4 activity. Fold-induction was calculated as the increase in expression of the treated samples relative to the non-treated control samples.

Histological analysis. Tissues were fixed in 10% buffered formalin, paraffin-embedded, and subjected to standard histochemical analysis. In some experiments, tissues were snap frozen upon harvest and cryosectioned prior to histologic or immunohistologic analysis.

Figure 1. NovoGen MMX Bioprinter.

Results

Figure 2. 3D tissue geometries with relevant architecture and cellular features fabricated using the NovoGen Bioprinter.

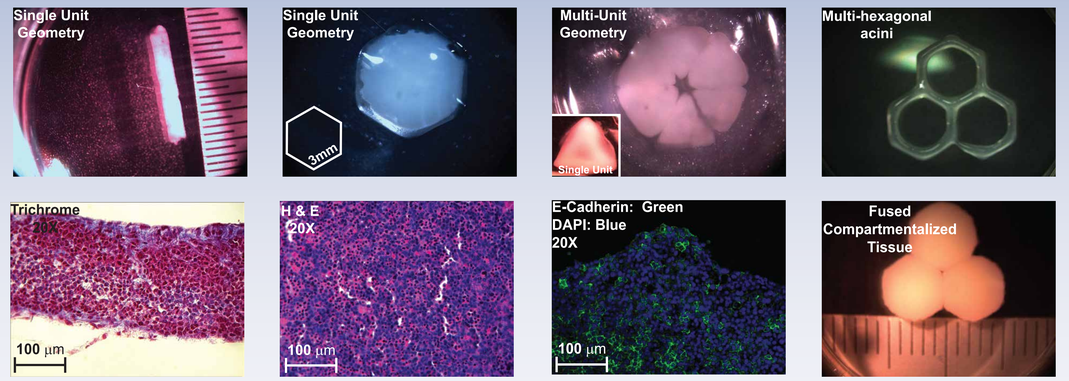
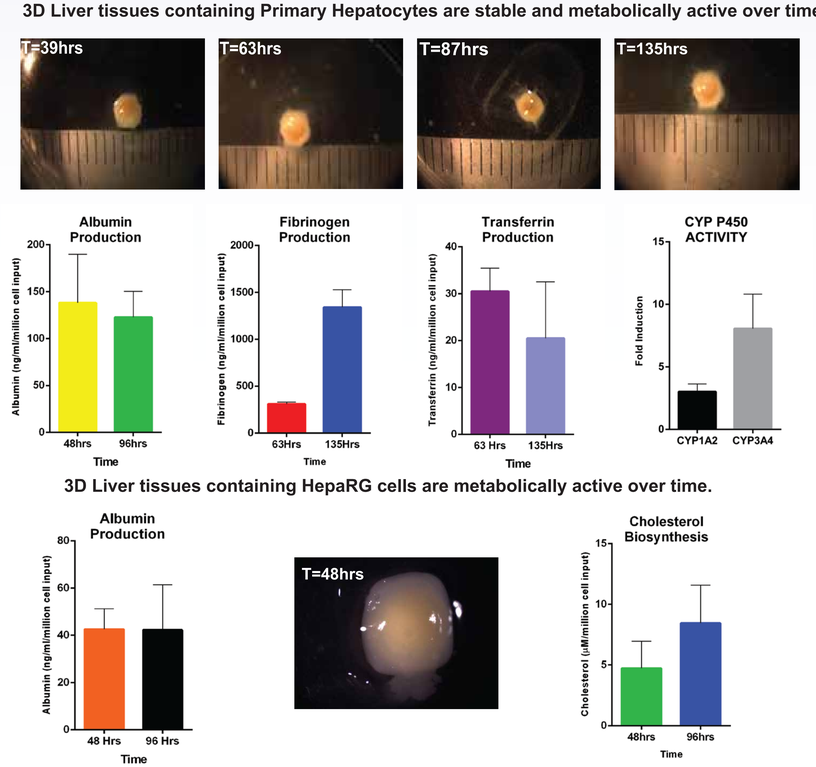
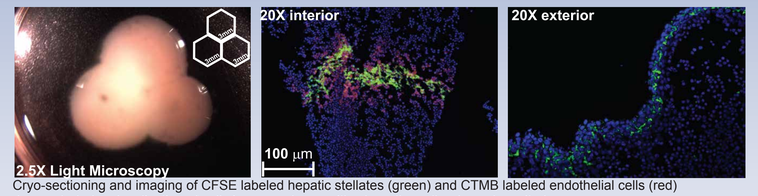


Figure 3. Bioprinted 3D human liver tissues constructed with primary hepatocytes and hepatic cell lines are metabolically active with CYP450 induction.



Results

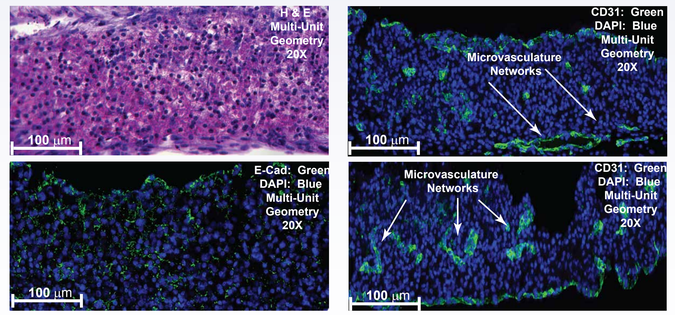
Figure 4. The NovoGen Bioprinter enables precise deposition of distinct cell populations within the bioprinted 3D liver tissue.



Cryo-sectioning and imaging of CFSE labeled hepatic stellates (green) and CTMB labeled endothelial cells (red) demonstrated co-localization of cells in concordance with where the cells where deposited.

Figure 5. 3D tissues bioprinted from iPSC derived hepatocyte-like cells outperform 2D culture.

Albumin production per cell was significantly enhanced in both multi-unit (p<0.001) and single unit (p<0.001) liver tissue compared to 2D monolayers.



Conclusions

Bioprinting enabled **highly reproducible** fabrication of architectually-and compositionally-defined 3D tissues into standard tissue culture formats.

Bioprinted 3D liver tissues exhibited several key features that remained stable over time:
1.) Tissue-like cellular density, with high viability and development of well-organized microarchitecture (microvasculature, tight junctions) indicative of substantial intercellular communication.
2.) Cell type-specific **compartmentalization**, with establishment and retention of user-defined spatial localization of parenchymal and non-parenchymal components.
3.) Multi-layered architecture, ranging from **250-500 microns** in thickness.

3D liver tissues possessed critical liver functions, including albumin production, cholesterol biosynthesis, fibrinogen and transferrin production, and inducible **CYP 1A2 and CYP 3A4 activities**.

Per cell protein production (Albumin) by 3D bioprinted liver tissues was **5.0-8.6**X greater than matched 2D controls, suggesting superior functionality in 3D.

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