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. Carbo- 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 thy-real-dimensional (3D) liver system that captures several key features of in vivo tissue, in a multi-well format suitable for drug screening. Using NovoGen\textsuperscript{TM} 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 liver metabolites: albumin, fibrinogen, and transferrin. Cholesterol biosynthesis was quantified using standard Organovo bioprinting protocols and the NovoGen MMX Bioprinter or modifications thereof. Bioprinting. All tissues were fabricated directly into standard tissue culture plates. (Corning Tissueware) using standard Organovo bioprinting protocols and the NovoGen MMX Bioprinter or modifications thereof. Sacred 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). CYP450 analysis. CYP1A2 and CYP3A4 activities were assessed with the Pro-Glo\textsuperscript{TM} CYP P450 Assay (Promega). Liver tissues were challenged with either variant (T454A) or dominant (T454M) 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 immunohistochemical analysis.

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.

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

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

Conclusions
Bioprinting enabled highly reproducible fabrication of architecturally-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 250-550 microns in thickness. 3D liver tissues possessed critical liver functions, including albumin production, cholesterol biosynthesis, fibrinogen and transferrin production, and inducible CYP1A2 and CYP3A4 activities. Per cell protein production (Albumin) by 3D bioprinted liver tissues was 5.0-6X greater than matched 2D controls, suggesting superior functionality in 3D.

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Figure 1. NovoGen MMX Bioprinter.

Figure 6. Bioprinted 3D human liver tissues constructed with primary hepatocytes and hepatic cell lines are metabolically active with CYP450 induction.