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 hepatoxicity 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 three-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.** Cells were purchased from commercially available sources. Standard cell culture was utilized for the propagation of non-parenchymal cell populations. CRYO-preserved primary hepatocytes were purifed by Percoll gradient centrifugation and cell viability was assessed by Trypan Blue exclusion. Thawing and maintenance of HepG2 cells was according to the manufacturer’s instructions.

**Preparation of bioprint and cell aggregates.** Bioprint and cell aggregates protocols were based on techniques described in Norotte et al., [2009].

**Preparation of hydrogels containing cells.** NovelGal™ and NovoGal 2.0™ (both from Organovo, Inc.) were prepared by manufacturers’ instructions. Non-parenchymal cell populations were incorporated into the hydrogels.

**Bioprinting.** All 3D tissue constructs were fabricated with NovoGen Bioprinters directly into standard tissue culture plates (CorningTreated) using standard Organovo bioprinting protocols.

**Metabolite detection.** Spent media was analyzed by commercially available ELISA kits for the following liver metabolites: albumin, cholesterol, fibrinogen, and transferrin.

**CYP450 analysis.** CYP1A2 and CYP3A4 activity was assessed with the Pro-Glo™CYP P450 Assay kit. Fold-induction was calculated as the increase in system (Promega). Liver neotissues were challenged with either verapamil (10µM) or dexamethasone (10µM) to stimulate CYP1A2 or CYP3A4 activity. Fold-induction was calculated as the increase in CYP P450 analysis.

**Results**

**Figure 1.** NovoGen MMX Bioprinter

**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 neotissue.

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

**Conclusions**

Bioprinting enabled highly reproducible fabrication of architecturally-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 (micromass, microtolam, 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 to 500 microns in thickness at the time of functional evaluation.

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 4-10x greater than matched 2D controls, suggesting superior functionality in 3D.

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