Bioprinted Human Liver Tissue With iPSC-Derived Hepatocyte-Like Cells

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Abstract

Use of iPSC allows for development of patient- and/or disease-specific in vitro systems, and may provide a means of generating cells that are difficult to isolate or propagate. For example, primary hepatocytes are routinely used in in vitro screens to predict hepatotoxicity; a primary reason for late-stage clinical failures and post-market withdrawals. We have previously reported successful fabrication and characterization of 3-dimensional (3D) human liver tissues, patterned with parenchymal (hepatocyte) and non-parenchymal regions (endothelial cells (EC) and hepatic stellate cells (hSC)), and have observed functional superiority of hepatocytes within those tissues. Here, we demonstrate feasibility that iPSC-derived hepatocyte-like cells (iPSC-HC) (iCell Hepatocytes, Cellular Dynamics Inc.) can be utilized as a potential alternative to primary hepatocytes in patterned 3D tissues. Within a 24-well plate, liver tissues with initial dimensions of 3mm x 3mm x 0.75mm were fabricated with the Novogen™ Bioprinting platform, comprising fields of iPSC-HC bordered by EC and hSC. Tissues were cultured for at least 7d, remaining viable and structurally stable. Biochemical analysis revealed liver-specific metabolites as early as 24h, which were detected for at least 7d. Histologic analysis at greater than 7d revealed a well-organized architecture, with E-cadherin-positive junctions between iPSC-HC and lumenized, CD31-positive microvessels. There was no evidence of necrosis, while limited cell proliferation (Ki67+) was observed. Taken together, although they do not contain the full repertoire of function or gene expression of primary hepatocytes, these data support continued investigation into the potential use of iPSC-HC in the fabrication of 3D liver tissues for drug discovery, development, and disease modeling.

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™ Bioprinter or modifications thereof.

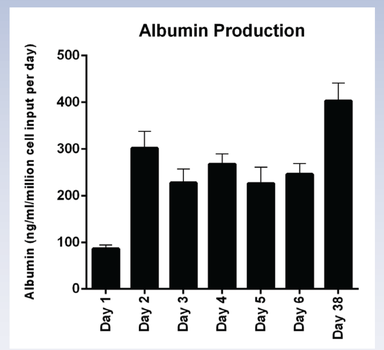
Secreted protein detection. Spent media was analyzed by a commercially available ELISA kit for the liver specific metabolite albumin. Cholesterol biosynthesis was quantified fluorometrically (Cayman Biochemical).

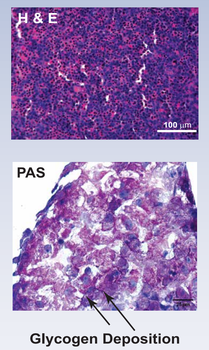
Histological analysis. Tissues were fixed in 10% buffered formalin, paraffin-embedded, and subjected to standard histochemical analysis. In some experiments, tissues were snap frozen upon harvest and cryosectioned prior to histologic or immunohistologic analysis.

Figure 1. Novogen Bioprinter

Results

Figure 2. 3D liver constructs with human liver cells are metabolically active over extended time points





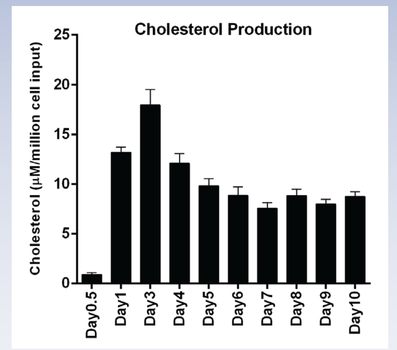
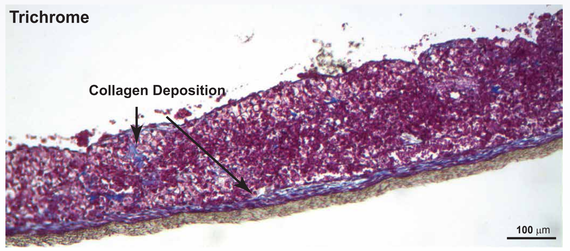
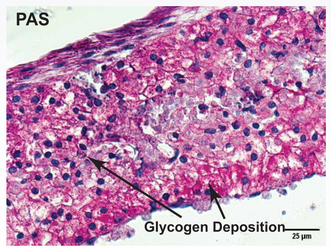
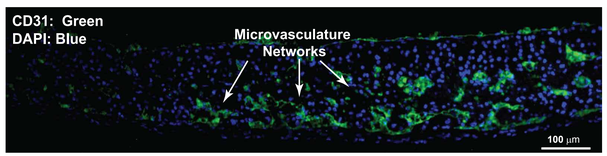


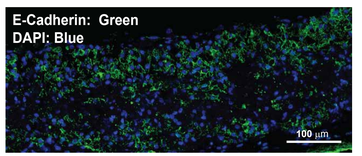
Figure 3. iPSC-derived hepatocytes result in cohesive tissues following bioprinting

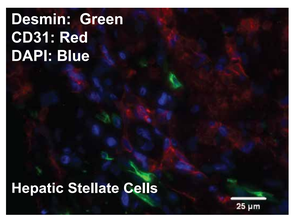
Histological Analysis

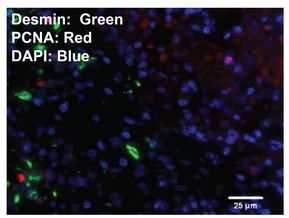


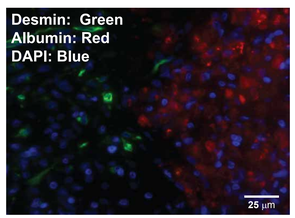








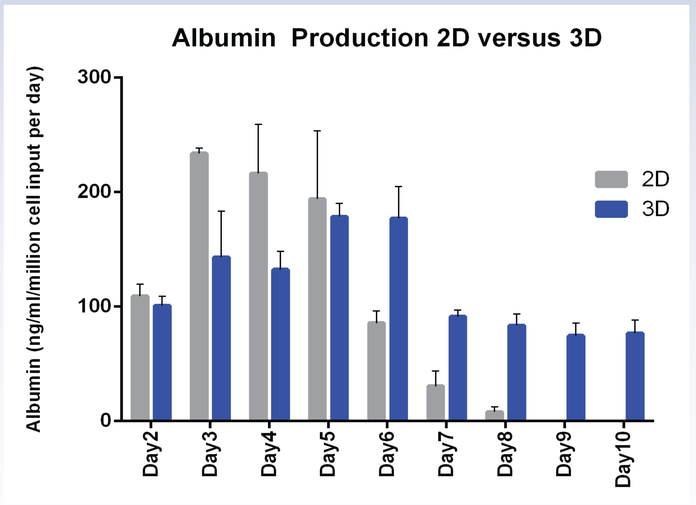




Results

Figure 4. iPSC derived hepatocytes bioprinted in 3D produce liver specific metabolites at extended time points

24 Well Format



Conclusions

Although not as metabolically active as the primary hepatocyte-containing constructs, the iPSC-derived hepatocytes yielded architectually-and compositionally-defined 3D tissues in standard tissue culture plates.

The bioprinted iPSC-derived 3D liver tissues exhibited several key features that remained stable over time including:

1) Reproducible tissues that were greater than 250 microns in thickness
2) High viability
3) Development of well-organized microarchitecture (microvasculture, tight junctions) indicative of substantial intercellular communication
4) Multiple liver relevant cell populations, which could be visualized by immunohistochemistry
5) Liver specific albumin production and glycogen storage
6) Production of albumin for longer time points than iPS cells plated in a monolayer

Acknowledgements

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Safe Harbor Statement

Any statements contained in this presentation that do not describe historical facts may constitute forward-looking statements as that term is defined in the Private Securities Litigation Reform Act of 1995. Any forward-looking statements contained herein are based on current expectations, but are subject to a number of risks and uncertainties. The factors that could cause actual future results to differ materially from current expectations include, but are not limited to, risks and uncertainties relating to the Company's ability to develop, market and sell products based on its technology; the expected benefits and efficacy of the Company's products and technology; the availability of substantial additional funding for the Company to continue its operations and to conduct research and development, clinical studies and future product commercialization; and, the Company's business, research, product development, regulatory approval, marketing and distribution plans and strategies. These and other factors are identified and described in more detail in our filings with the SEC on March 15, 2013, our transition report on Form 10KT filed with the SEC on May 24, 2013, our quarterly report file on Form 10Q with the SEC on August 9, 2013 and our current reports filed on form 8K. We do not undertake to update these forward-looking statements made by us.