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Spotlight

A Bioprinted Liver-on-a-Chip for Drug Screening Applications

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The need for a liver-on-a-chip tissue model for drug screening is particularly important in tissue engineering because of the high frequency of drug-induced liver injury. Recently, a liver tissue model conducive to hepatotoxicity testing was developed by bioprinting hepatic spheroids encapsulated in a hydrogel scaffold into a microfluidic device.

Motivation for Liver-on-a-Chip Platforms

Pharmaceutical development is costly (e.g., $2.6 billion per drug that enters the market, csdd.tufts.edu/news/complete_story/pr_tufts_csdd_2014_cost_study) and inefficient (e.g., 94% of drugs fail in the clinical trial phases, www.fda.gov/ForPatients/Approvals/Drugs/ucm405622.htm). This is partially due to inadequate screening in preclinical trials, which can be remediated using 3D engineered tissue platforms. 3D engineered tissues can mimic the *in vivo* structure, function, and response to drugs of human tissues more accurately than 2D models, leading to better *in vitro* efficacy and safety screening before human trials [1,2]. Bioprinting has shown promise in creating complex 3D cellular architectures for a wide range of applications [3–6]. Microfluidic systems have been explored as a promising platform for high-throughput assays offering the ability to precisely control the flow of fluid, provide nutrients, oxygen, and growth factors, as well as remove waste, and have been shown to facilitate the creation of several organs-on-a-chip including cardiac [7] and skeletal muscle [8] and cancer tissues [9,10].

Recently, a liver-on-a-chip platform has been developed by Bhise *et al.* with hepatic spheroids fabricated via direct write bioprinting in a microfluidic bioreactor device [11]. This liver tissue model is an important advance in tissue engineering for drug screening applications. Drug-induced liver injury is a serious concern during pharmaceutical development and is the most common cause for discontinuing clinical trials and for withdrawal of approved drugs during the postmarket surveillance stage (http://www.fda.gov/ScienceResearch/BioinformaticsTools/LiverToxicityKnowledgeBase/ucm2024036.htm). In the model, liver tissue is printed directly into the microfluidic device, which is then assembled around the bioprinted tissue and serves as a bioreactor to maintain long-term viability. Further, the device can be easily disassembled and reassembled to allow access to the cells over the course of the experiment, which is uncharacteristic of most microfluidic devices used for tissue engineering. This microfluidic platform can serve as a 3D culture environment to culture liver tissues and test for drug-induced toxicity with high throughput *in vitro*.

Bioreactor Platform Features

The bioreactor platform presented in this study was fabricated with multiple layers of polydimethylsiloxane (PDMS) and poly (methyl methacrylate) (PMMA). Unlike typical microfluidic devices, this platform was designed to be opened to allow direct access to the microtissues during the experiment. Following fabrication, the device was perfused with cell media at a rate chosen to provide sufficient oxygen and nutrients to the microtissues without diluting the biomarkers secreted by the cells, which could interfere with cellular behavior and response to drugs and toxins (Figure 1A, C) [11]. This system is unique in that it is able to incorporate both bioreactor-like perfusion as well as direct-write bioprinting.

Bioprinting Approach to Liver-on-a-Chip Fabrication

HepG2/C3A human hepatocarcinoma cells were formed into 191 μm (±10 μm) spheroids using a microwell technique (the spheroids were reported to be small enough to avoid necrosis but superior to monolayer cultures in functionality). These spheroids were suspended in gelatin methacryloyl (GelMA), a bioresponsive, degradable, natural extracellular matrix (ECM)-derived hydrogel scaffold with 0.5% photoinitiator. A modified version of an Organovo NovoGen MMX bioprinter™ was used, which functions by extruding the mixture through a hollow capillary using a piston system while exposing to UV light to crosslink the hydrogel. An automated X-Y-Z stage was used to deposit liquid droplets of the mixture in a 7 × 7 array directly into the device (Figure 1B). The technique by Bhise and colleagues represents an interesting approach to organ-on-a-chip fabrication in which bioprinting has enabled patterning of hepatic spheroids contained within 3D hydrogel constructs directly into the microfluidic device. Hepatic spheroids have been previously shown to facilitate more biomimetic activity in hepatic cells and culturing them in a 3D microenvironment within a microfluidic device will allow more convenient application of these cells to drug screening applications [11].

Viability and Drug Toxicity Screening

Following fabrication, the number of cells increased 10-fold over a 30-day incubation period. Bioactivity was sustained over this time period in terms of biomarker secretion and expression patterns of intracellular proteins. Drug toxicity was also tested using an acute toxic dose of acetaminophen (the LC_{50} value determined under static conditions) by continuous exposure over 1 week. Results show a significant decrease in metabolic activity over 6 days in cultures with acetaminophen; decreases in cell density, activity, and biomarker production were also observed (Figure 2). These results are similar to the

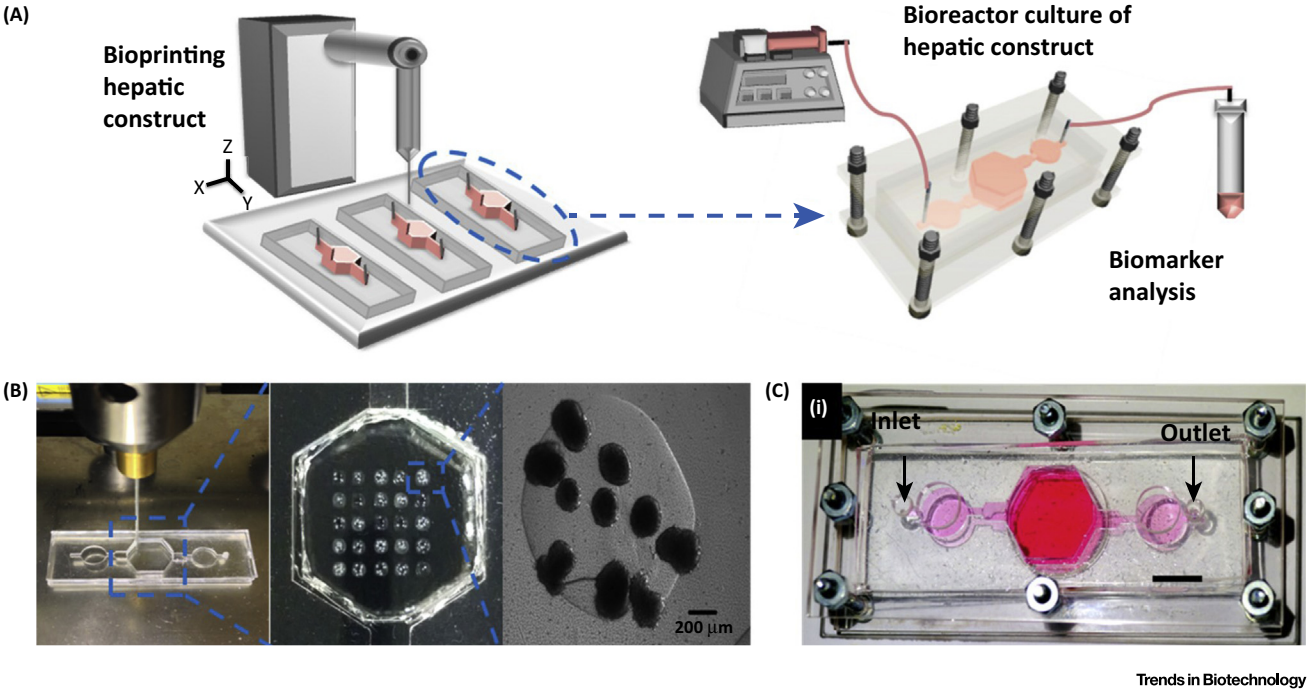

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Figure 1. Liver-on-a-Chip Platform Fabrication. (A) Schematic of bioprinting of microtissue constructs directly into the device, followed by assembly of the bioreactor chip and perfusion of cell media. (B) Direct-write bioprinting of GelMA hydrogel constructs as a dot array with encapsulated hepatic spheroids. (C) Top view of the bioreactor device assembled using screws; the inlet and outlet for flow of cell media and the cell culture chamber are shown. Reproduced, with permission, from [11]. (© IOP Publishing. All rights reserved.)

acetaminophen-induced hepatotoxicity reported in animal and *in vitro* models [11].

Conclusions

The liver tissue model presented here is an important advance in tissue engineering for drug screening applications. Drug-induced liver injury is a serious concern during pharmaceutical development and is the most common cause of discontinuing clinical trials and for withdrawal of approved drugs during the postmarket surveillance stage (http://www.fda.gov/ScienceResearch/BioinformaticsTools/LiverToxicityKnowledgeBase/ucm2024036.htm). Facing a pressing commercial need for better drug screening in the preclinical trial phase, this platform offers a useful tool to better recapitulate the *in vivo* structure, function, and drug reactions of human tissues. We highlight promising results showing long-term culture of bioprinted hepatic spheroids as well as drug toxicity testing using acetaminophen as a model drug [11]. The microfluidic platform used here is conducive to automated testing of multiple drugs and cells from multiple patients in an efficient, high-throughput manner. A cell line was used as an accessible and reliable cell source; future studies using hepatocytes derived from induced pluripotent stem cells (iPSCs) may extend the clinical application to cells from multiple patients, thus capturing the population variability needed to better screen drugs before human clinical trials. This approach can ultimately help to detect and elucidate the cause for idiosyncratic drug-induced liver injuries which are rare adverse drug interactions and are currently difficult to predict in clinical trial phases. In future studies, advanced 3D bioprinters may be utilized to print and fabricate both the microfluidic platform and patterned complex tissues inside the device simultaneously, which will greatly simplify the fabrication of organ-on-a-chip models.

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References

1. Griffith, L.G. and Naughton, G. (2002) Tissue engineering – current challenges and expanding opportunities. *Science* 295, 1009–1014
2. Wobma, H. and Vunjak-Novakovic, G. (2016) Tissue engineering and regenerative medicine 2015: a year in review. *Tissue Eng. Part B Rev.* 22, 101–113
3. Tasoglu, S. and Demirci, U. (2013) Bioprinting for stem cell research. *Trends Biotechnol.* 31, 10–19
4. Knowlton, S. *et al.* (2015) Bioprinting for cancer research. *Trends Biotechnol.* 33, 504–513
5. Kang, H.W. *et al.* (2016) A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nat. Biotechnol.* 34, 312–319
6. Durmus, N.G. *et al.* (2013) Bioprinting: functional droplet networks. *Nat. Mater.* 12, 478–479
7. Aung, A. *et al.* (2016) 3D cardiac mutissues within a microfluidic device with real-time contractile stress readout. *Lab. Chip* 16, 153–162
8. Shimizu, K. *et al.* (2015) Microfluidic devices for construction of contractile skeletal muscle microtissues. *J. Biosci. Bioeng.* 119, 212–216
9. Gao, D. *et al.* (2010) A microfluidic approach for anticancer drug analysis based on hydrogel encapsulated tumor cells. *Anal. Chim. Acta* 665, 7–14
10. Sung, J.H. *et al.* (2010) A microfluidic device for a pharmacokinetic-pharmacodynamic (PK-PD) model on a chip. *Lab. Chip* 10, 446–455
11. Bhise, N.S. *et al.* (2016) A liver-on-a-chip platform with bioprinted hepatic spheroids. *Biofabrication* 8, 014101