Bioprinted liver organoid for toxicology testing

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Introduction

Recently, body-on-a-chip technology has gained attention as a viable option to replace animal experimentation in drug discovery.[1] These chips generally consist of tissue organoids, microfluidics, and biosensors designed to analyze the toxicity and effectiveness of new drug candidates. In this study, we have developed 3D liver organoid constructs using bioprinting technology to produce computer designed 3D structures containing primary human hepatocyte organoids embedded within a functionalized hydrogel bioink. Steady state functionality of the bioprinted liver organoids was confirmed under normal conditions. Additionally, the expected cytotoxic response to acetaminophen (APAP) overdose was observed in toxicology assays.

Material and methods

A liver extracellular matrix (ECM)-based hydrogel was developed as a bioink for bioprinting of the liver organoids.[2] The ECM solution was obtained by combining dissolved decellularized liver tissue with heparin conjugated hyaluronic acid (Extracel™-HP of Glycosan Biosystems) that contained a photo-initiator. Liver spheroids (300µm diameter) composed of primary human hepatocytes were suspended in the bioink. Polycaprolactone (PCL) was co-printed with the bio-ink to stabilize the hydrogel structure. The bioink was printed with a 400µm nozzle under 20Kpa at 18°C. PCL was printed using a 300µm metal nozzle at 760Kpa, and 96°C. The constructs were printed into a microfluidic system and cured with UV light. Viability and function of the bioprinted organoids were determined at 14 days under flow conditions. Acetaminophen (APAP) was applied to the organoids for toxicology testing.

Results

Fig. 1(a) shows the design and patterning of the bioprinted liver organoid/PCL constructs. Fig. 1(b) shows live/dead staining of the organoid at 14d. The bioprinted structures were maintained under flow condition and cell viability was largely preserved throughout the study. Albumin and urea secretions were also maintained for 14d, confirming the functional stability of the bioprinted constructs (Fig. 1(c)). A sharp decrease in organoid function was seen following intoxication by 1 and 10 mM APAP. These doses are within the toxic range for APAP in human serum, demonstrating a physiologically appropriate response to drug induced injury.

Conclusion

In this research, bioprinting technology was used to produce liver organoid constructs. The results showed that liver organoids maintained viability and function for 14d under flow conditions following bioprinting. Additionally, the bioprinted liver organoids responded appropriately to APAP intoxication; verifying the utility of this platform for drug discovery and toxicity testing. These results validate the application of bioprinting technology for the production of liver organoid constructs for drug studies. Future works will focus on determining the effect of construct micro-architecture and cell type composition on long term liver organoid viability and function.

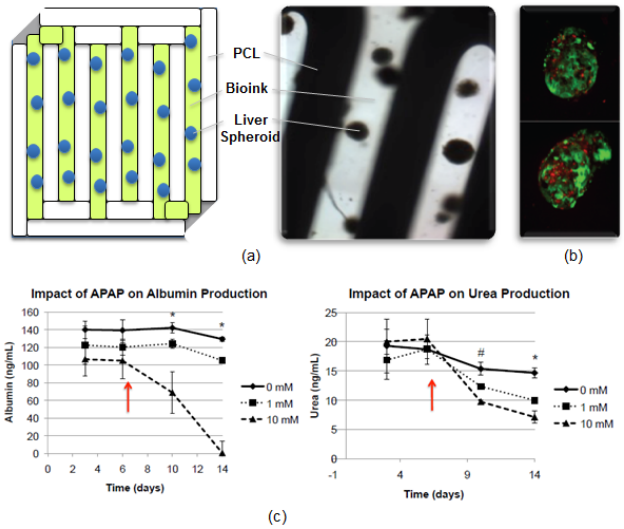

Fig. 1 Bio-printed liver organoids: (a) schematic diagram and microscope image of bioprinted structure, (b) confocal microscope image of bioprinted liver organoids after live/dead staining (live: green, dead: red), and (c) functionality test results under normal and toxic conditions.

References

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2. A. Skardal et al. Tissue specific synthetic ECM hydrogels for 3-D in vitro maintenance of hepatocyte function. Biomaterials 33:4565-4575, 2012.

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