Editorial

Advances in 3D bioprinting technology for liver regeneration

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The liver is involved in over 500 biochemical reactions. Over the last couple of decades, liver diseases have become a major cause of morbidity and mortality in the world (1). Unfortunately, treatment options for patients with chronic liver disease remain limited. Due to the lack of highly sensitive and effective detection methods, most patients with liver cancer are already in an advanced stage when diagnosed (2). As the only effective way to cure patients with chronic and acute liver failure, liver transplantation has a severe shortage of organ donors, and the surgery is therefore costly. Additionally, a significant number of patients have postoperative immune rejection (3).

The progression of research in this field is hindered by the lack of suitable cell and animal research models. Currently, research models for liver regeneration mainly include mouse models, organoids, and 2D cells. As one of the most commonly three-dimensional (3D) disease models, patient-derived xenograft (PDX) models have drawbacks, including ethical controversy, lengthy protocols, high cost, unsuitability for testing of immunotherapies, and complicated operation. Lacking of key vascular network and immune system composed of kinds of stromal cells, another model, organoids, is also not the perfect model of primary cells. Conventional cell lines undergo an adaptive transformation during long-term culture *in vitro*, resulting in significant changes from real cells *in vivo*. Primary cells lose many of their inherent features in the 3D environment *in vivo*. Therefore, 2D cell culture models cannot reproduce the real microenvironment in a tissue or organ *in vivo*, even present misleading results (5).

As an emerging interdisciplinary frontier technology, 3D bioprinting can control the spatial arrangement of cells and the microenvironment around them, so it has a great potential and application prospects in tissue and organ construction. Cells, hydrogels, and other factors are arranged to form tissue-like 3D structure, which can promote cells to express *in vivo* functions. Over the past decade, 3D bioprinting has provided various strategies for building biologically functional tissues. Bioprinting of various cells is at the forefront of the 3D printing field and has been applied to the *in vitro* reconstruction of tissues and organs, such as the heart, blood vessels, and lungs, which may help alleviate the organ shortage crisis (6). Furthermore, 3D bioprinting recapitulates liver functions to better suit research purposes.

Initially, people attempted to construct a 3D microenvironment with the bioprinting technique by encapsulating hepatocytes (HCs) into biomaterials. Researchers constructed a scaffold model with gelatin and seeded HUH7 cells. They then tested liver-specific functions and found that HUH7 demonstrated high viability and proliferation in a 3D-bioprinted environment (7). Although Huh-7 is an undifferentiated HC, to the best of our knowledge, this is a hepatocellular carcinoma cell line. Later, researchers changed their focus from one type of cell to multicellular bioprinting to better mimic hepatic functions.

Multicellular bioprinting is an emerging area in 3D bioprinting, and vascularization is one of the main considerations in liver models using this technique. With the development of printing techniques, the vascular system can now be embedded into models created by 3D-bioprint technique. Researchers successfully constructed hepatic lobules by 3D-bioprint technique. The lumen in the center of the hepatic lobules representing the portal vein *in vivo*, cells surrounding it including HCs and

endothelial cells surrounding them (8). Organovo™, one of the primary companies involved in 3D bioprinting, has successfully generated 3D liver tissue mimetics containing architecturally- and physiologically-relevant features. Using this technology, Presnell *et al.* constructed 3D structures that highly mimic liver lobules *in vivo*. Cell embedded in the construction including HCs, endothelial cells, and hepatic stellate cells. They also showed high viability and long-term functionality (9). People used primary rat HCs, endothelial cells, and fibroblasts to construct a 3D model for liver tissue engineering. Then the viability and functions of HCs in the printed liver construct were improved by interactions among cells induced by 3D bioprinted microenvironment (10). These studies were important, as they showcased the significant capacity of 3D bioprinted construction with a capillary-like network for *in vitro* liver tissue construction.

Combined with other cutting-edge cytology research results, 3D bioprinting has presented exciting results. Yang *et al.* bioprinted liver tissue models via digital light processing and embedded human-induced HCs in models that expressed excellent HCs functions (11). Hepatic progenitor cells were used to construct a 3D-bioprinted hepatorganoids model in our research. These hepatorganoids showcased *in vivo* hepatic function, as well as an ability to relief symptom of liver failure while transplanted into mice. These results showed the 3D-bioprinted hepatorganoids were available for liver regeneration (12). Other researchers printed human-induced pluripotent stem cells and human embryonic stem cells with a bioprinter, which could be induced differentiation into hepatocyte-like cells. Cells bioprinted in 3D construction displayed remarkable results for secreted proteins associated with liver function and were suitable for the generation of livers-like constructions as the drug-testing models (13).

Though constructions based on 3D bioprinting technology can mimic the microenvironment of liver *in vivo*, these models still have many shortcomings. Cells in constructions are usually statically immersed in the medium, and it is difficult to refresh the gas and nutrients. Further work must be performed before the liver construction can be successfully used in clinic, such as the *in vivo* compatibility, toxicity and degradation rate of hydrogel scaffolds. In addition, the cells used for 3D bioprinted liver tissue are predominantly cell lines, human-induced pluripotent stem cells, and human embryonic stem cells, which differ from primary hepatocytes. It has also been reported that hepatocytes can be transformed and cultured *in vitro*, but the future use of this type of cell will inevitably cause concerns (14).

Isolating liver tissue and culturing primary hepatocytes *in vitro*, combined with constant optimization of 3D bioprinting, will have great potential in preserving the transcriptional properties and biological activities of hepatocytes (15). This model also has enormous implications in the fields of regenerative medicine, personalized drug testing, and liver disease research.

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