Editorial

Highlights of constructing liver-relevant *in vitro* models with 3D bioprinting

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The liver plays a key role in metabolism, digestion, detoxification, and other physiological processes, and thus liver-related diseases pose a considerable threat to global health. Therefore, exploring the mechanisms and pathophysiology of these diseases is of great clinical significance. In addition, the shortage of donors limits the application of liver transplantation in certain cases of end-stage liver diseases (1,2). Manufacturing artificial liver tissue with physiological function using tissue-engineering technologies may be a solution to partially replacing liver function or a potential source of liver transplantation (3). Three-dimensional (3D) bioprinting is a commonly used tissue-engineering technology for fabricating preset macrostructures with cell-loading bioinks. According to the working mechanisms, 3D bioprinting can be divided into inkjet-based bioprinting, photocuring-based bioprinting, and extrusion-based bioprinting. Among various tissue-engineering technologies, 3D bioprinting has attracted wide attention and achieved significant progress in recent years. Herein, we review the highlights of the latest progress, the notable research hotspots, and the future prospects of 3D bioprinting in liver-related preclinical studies from the aspects mentioned above.

An ideal bioink should possess excellent printability and shape fidelity to support cell growth and form a precise structure. The biocompatibility of bioinks is also crucial for promoting cell growth, maintaining cell function, and supporting subsequent analysis. In current 3D bioprinting systems, the commonly used components of bioinks include alginate, gelatin, collagen, decellularized extracellular matrix (dECM), fibrin, fibroin, and methacrylated gelatin (GelMA) (4). A desired biofabrication window can be achieved by blending different components and by adjusting the parameters of bioprinting. For example, blending solubilized native decellularized liver matrix and silk fibroin has been reported to promote the proliferation of liver cells and improve their biological function (5). Similarly, it has been reported that dECM can improve the viability and function of certain processes, such as in the albumin and urea secretion of human-induced hepatocytes (hiHep cells) (6). To construct complex structures, bioinks can be combined with sacrificial materials. Sacrificial bioinks are printable biomaterials that can be printed together with other bioinks and that gradually dissolve during subsequent culture. Blending sacrificial bioinks with other bioinks can be used to construct porous structures, while printing the two bioinks separately through multinozzle bioprinting can be used to build complex structures, such as perfusable channels. There are numerous reports on the construction of *in vitro* models using different bioinks, which are not discussed here in detail. As new bioinks continue to emerge, there remain questions concerning the exact effect of individual bioinks on embedded cells and the optimal bioink for each cell type and its proper concentration. Exploring the effects of different bioinks and their concentrations on the viability and function of hepatocytes and interstitial cells may enable us to develop novel bioinks.

Hepatic lobules are the fundamental structures and functional units of liver tissue. Due to its small size and complex structure, it is a challenging issue for tissue engineering to restore the structure and function of hepatic lobules *in vitro*. To address this issue, digital

light stereolithography has been applied to achieve high resolution in 3D bioprinting. For example, biliary epithelial branched networks have been successfully constructed to simulate the structure of bile ducts in the liver (7). Furthermore, digital light stereolithography has been used to construct microscale hexagonal architecture containing hepatic cells, endothelial cells, and adipose-derived stem cells (8). Apart from digital light stereolithography, the usage of a precursor cartridge is also a solution for improving the resolution of 3D bioprinting. Specifically, precursor cartridges with the same shape as the structure of interest can be placed in the extrusion channel, and the compartments can be filled with different bioinks to achieve the regulation of microstructure. This process, through the usage of precursor cartridges together with a microfluidic system, has been reported to be capable of constructing a microstructure similar to liver lobules with a resolution of 20 μm, which is much higher than that achieved with traditional 3D bioprinting (9). This microscale-to-macroscale biomanufacturing method can be easily combined with extrusion 3D bioprinting, which has promising application prospects. However, existing 3D bioprinting methods with improved resolution often have a limited potential in high-throughput applications. The tradeoff between printing accuracy and potential in high-throughput application is key to creating a sound workflow for different research purposes.

Appropriate model designing and selection of cells loaded in bioinks are vital for simulating the physiological environment of the liver. Since uniform cell components cannot restore the structure of the liver and since 3D bioprinting allows the manufacture of different cell types, the construction of multicellular, multicomponent and perfusable *in vitro* models is one of the hotspots in 3D bioprinting. A perfusable multicellular model containing blood vessels combined with a microfluidic system has been reported on, in which the innermost sacrificial bioink created the lumen of the vascular structure. Bioink composed of GelMA and fibrin was loaded with HepG2 and other cells to mimic liver function. The outer layer composed of poly(dimethylsiloxane) (PDMS) provided sufficient mechanical strength and was used to connect microfluidic devices or blood vessels (10). This well-designed model served as a proof of concept that 3D-bioprinted tissue can be used as a vascularized graft with the potential as a functional substitute for liver tissue in the future, thus prolonging the survival of patients with end-stage liver diseases (11). As for the selection of cells, liver is composed of hepatocytes, endothelial cells, Kupffer cells, fat-storing cells, and liver stem/progenitor cells. According to the physiological environment and cell composition of liver, optimizing the types, distribution, and proportion of cells is crucial in model development. Furthermore, a 3D-bioprinted model can be employed in drug screening to minimize animal use and provide precise pathological knowledge of diseases. Screening antitumor drugs using patient-derived tumor is another important clinical application of 3D bioprinting. For example, patient-derived intrahepatic cholangiocarcinoma cells have been used for tumor modeling and drug screening (12). 3D-bioprinted patient-derived tumor models are expected to become a reliable method for drug screening and efficacy prediction, providing valuable information for the selection of clinical therapies in the future.

In summary, 3D-bioprinted liver tissues can mimic the microenvironment of the liver and serve as a research model to facilitate mechanism exploration. 3D bioprinting can also construct artificial tissues as potential sources of grafts. In addition, the 3D-bioprinted models can be applied to drug development, antitumor drug screening, and personalized medicine (13). Although 3D bioprinting is widely used and significant progress has been made, limitations exist that will inform the directions of future research. In current 3D-bioprinted patient-derived tumor models, the limited number of cells derived from tissue sample hinders its application in personalized medicine. Unfortunately, this drawback also makes it difficult to construct 3D-bioprinted models from biopsy samples in guiding the selection of neoadjuvant therapies and downstaging strategies in clinical practice (14). In terms of postprinting culture and analysis, exploring the combination of other downstream analysis methods and 3D bioprinting systems may be prove vital and greatly facilitate further research in liver-related diseases (15). In conclusion, applying 3D bioprinting and other tissue-engineering technologies to construct *in vitro* tissues mimicking the native liver environment is of great significance and warrants further exploration.

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