Editorial Commentary

Advances in the construction of *in vitro* liver tissue models using 3D bioprinting technology

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The liver, being the largest solid organ in the human body, performs a multitude of essential physiological functions, including digestion, metabolism, detoxification, and protein synthesis (1). Impairment of liver function due to disease or surgical intervention can result in a reduction of functional liver volume, with potentially severe or even life-threatening consequences (2). The construction of these liver tissue models facilitates the investigation of the liver's intricate physiological functions and holds considerable clinical relevance in numerous fields, including regenerative medicine, drug metabolism, toxicology research, and drug efficacy screening. In recent years, the emergence and advancement of three-dimensional (3D) bioprinting technology has led to significant progress in the development of functional liver models *in vitro*.

In order to accurately replicate the physiological characteristics of the liver *in vitro*, it is essential to closely simulate the composition and microstructure of liver tissue as it exists *in vivo*. The liver lobule represents the most fundamental structural unit of the liver, with liver tissue itself comprising over 80% hepatocyte parenchyma and the remaining stroma. The cellular constituents of the liver parenchyma encompass both hepatocytes and bile duct cells, while the stroma is composed of a diverse array of cells, including sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells (fat-storing cells; FSCs), and pit cells (3). The liver's various physiological functions related to synthesis and metabolism are inextricably linked to the complex interplay between these various components. 3D bioprinting technology enables the precise assembly of biocompatible materials and biologically active materials such as cells, thereby providing a means by which to accurately replicate the intricate structural composition of the liver *in vitro*. Based on the working mechanisms, 3D bioprinting can be classified into three categories: extrusion-based, droplet-based, and photocuring-based bioprinting (4). A wide range of biomaterials have been developed to fulfill various performance criteria, including suitable mechanical strength, excellent biocompatibility, bioactivity, gentle crosslinking conditions, and the feasibility of sacrificial dissolution. These biomaterials encompass, but are not limited to, substances such as gelatin, alginate, collagen, decellularized extracellular matrix, fibrin, fibroin, and gelatin methacrylate (GelMA) (5).

Yang *et al.* utilized extrusion-based 3D bioprinting technology to construct 3D bioprinted hepatorganoids (3DP-HOs) using the HepaRG cell line. Following 7 days of *in vitro* differentiation with dimethyl sulfoxide (DMSO), the 3DP-HOs extensively acquired a range of liver functions, including albumin secretion, drug metabolism, and glycogen storage. When transplanted into mice exhibiting liver function defects, the 3DP-HOs successfully prolonged the animals' survival time. Accompanying the emergence of human-specific drug metabolism activity, the liver function indicators of the mice increased and vascularization of the 3DP-HOs was observed (6).

With the ongoing advancement of 3D bioprinting technology, multicellular printing has emerged as a novel

trend. It is now extensively utilized to investigate the crucial role of the liver lobule microenvironment (7). Cuvellier *et al.* utilized a combination of the HepaRG cell line, the human hepatic stellate cell line LX-2, and human umbilical vein endothelial cells (HUVECs) to conduct co-printing. Their observations revealed that the LX-2 cell exhibited significant expression of fibroblast genes during co-culture, with collagen deposition occurring exclusively within this context. These findings may serve to provide new insights into the molecular and cellular mechanisms underlying liver fibrosis (8). Janani *et al.* used a combination of hepatocyte-like cells (HLCs) [derived from adipose-derived stem cells (ADSCs) differentiation], HUVECs, and human hepatic stellate cells in their research. Their observations similarly indicated that the presence of a complex cellular composition served to enhance both cell proliferation activity and liver function (9).

With the advancement of research, traditional extrusion-based 3D printing technology is no longer sufficient to meet the precision requirements necessary for accurately reproducing tissue microstructures. Consequently, a growing number of innovative printing methods are being developed. Ma *et al.* utilized digital light processing (DLP) 3D printing technology to solidify two types of bioinks in solution through numerically controlled light exposure, according to a specific spatial structure. This formed high-precision central liver parenchymal hexagons, as well as interstitial and endothelial cell structures located in the interlobular space. In this model, the stroma, constructed with ADSC and HUVEC, enhanced the proliferation vitality and liver function of liver parenchymal cells derived from induced pluripotent stem cells (iPSCs) differentiation (10). Hong *et al.* proposed a technique for producing structured microtissue spheroids through the use of coaxial extrusion and emulsification truncation. By employing a thin layer of bioink containing the endothelial cell line EA.hy926, they were able to effectively separate the tissue bioink containing HepG2/C3A cells, thereby simulating the structure of liver lobules. When compared to non-structured spheroids produced by mixing two types of cells, these structured spheroids featuring lobules exhibited a more stable morphology, higher levels of differentiation and maturation during long-term culture, and increased vascularization and cell density following transplantation (11). Indeed, the cell density of *in vitro* tissues constructed using existing 3D bioprinting technology remains at least one order of magnitude lower than that of *in vivo* tissues. In an effort to address this limitation, Fang *et al.* have proposed a novel method for preparing high cell density bioink. This process involves the aggregation of suspended cultured HepG2 cells into cell clusters comprising approximately 200 cells, which are then encapsulated with hydrogel to form granular cell aggregate-based biphasic (GCAB) bioink. When compared to bioink composed of dispersed cells at the same density, GCAB bioink printed bodies exhibit higher liver function activity. Furthermore, the addition of HUVECs for co-printing results in enhanced vascularization effects when using GCAB bioink (12).

It is of significance to note that primary human hepatocytes (PHHs) are commonly deemed incapable of sustaining prolonged *in vitro* culture under two-dimensional conditions. Nevertheless, Cuvellier *et al.* have demonstrated the feasibility of encapsulating PHH in a state of low aggregation through hydrogel printing, thereby characterizing the potential for PHH proliferation and differentiation within a 3D culture environment. The results of their study indicate that PHH cultured in 3D hydrogel exhibit consistent liver function activity in both *in vitro* and *in vivo* experimental settings (13).

In summation, 3D bioprinting, as an emergent technology, has been successfully implemented in the construction of *in vitro* liver tissue models. Current research indicates that 3D-printed liver tissue models exhibit considerable liver function activity both *in vivo* and *in vitro*. Multicellular modeling approaches employ interstitial cells, primarily comprising endothelial cells, stellate cells, and stem cells, to provide insight into the molecular and cellular mechanisms underlying vascularization and fibrosis processes. The construction of liver parenchyma primarily utilizes cells such as the immortalized liver progenitor cell line HepaRG, pluripotent stem cells, liver cancer cell lines, and other cell types that exhibit relative maturity under two-dimensional culture conditions. With technological advancements, PHH can now be cultured and proliferated under 3D conditions, representing a promising area of future research.

However, in comparison, the reliable replication of the liver's intricate structure remains a formidable challenge in current research. The most widely utilized extrusion-based 3D bioprinting technology suffers from the drawback of low resolution, rendering it difficult to reproduce the liver sinusoid and liver plate structures, which consist of only a single layer of cells within the liver lobule. Concurrently, extrusion-based 3D bioprinting technology inevitably inflicts shear damage upon the cells within the bioink, thereby limiting the survival rate and functionality

of printed tissue cells. Moreover, hydrogel matrices that differ from the *in vivo* environment can impede biological behaviors such as cell migration, extension, and proliferation, which can influence the self-assembly process of cells growing into tissues to a certain extent (14). As such, future research must concentrate on innovations in printing methods. For instance, acoustic tweezers technology can manipulate cells on a micrometer scale without contact, positioning them in designated locations within printed tissues with satisfactory precision and high biosafety (15). Acoustic tweezers technology is anticipated to become a revolutionary next-generation method for 3D bioprinting liver tissue *in vitro*. Additionally, the complex blood supply patterns and biliary system within liver lobules, as well as their intricate immune microenvironment, have yet to be effectively constructed *in vitro* models. Apart from higher printing precision, more complex co-cultured cell types, materials with greater biocompatibility, more sophisticated modeling concepts, and more dependable nutrient supply systems can all become future research directions. There remains a vast expanse for exploration in the scheme of liver tissue modeling *in vitro*.

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Footnote

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References

1. Kuntz E, Kuntz HD. Biochemistry and Functions of the Liver. Hepatology Textbook and Atlas. Berlin, Heidelberg: Springer Berlin Heidelberg; 2008:35-76.
2. Xing J, Wan X, Sang X, et al. Perioperative management of patients with liver cancer complicated with sarcopenia. Hepatobiliary Surg Nutr 2022;11:906-8.
3. Horst DA, Karpen SJ. Chapter 120 - Bile Formation and Cholestasis. In: Polin RA, Fox WW, Abman SH. editors. Fetal and Neonatal Physiology. 3rd edition. W.B. Saunders; 2004:1186-98.
4. He Y, Gu Z, Xie M, et al. Why choose 3D bioprinting? Part II: methods and bioprinters. Bio-des Manuf 2020;3:1-4.
5. Zhang J, Yang H, Yang H. Highlights of constructing liver-relevant in vitro models with 3D bioprinting. Hepatobiliary Surg Nutr 2022;11:896-8.
6. Yang H, Sun L, Pang Y, et al. Three-dimensional bioprinted hepatorganoids prolong survival of mice with liver failure. Gut 2021;70:567-74.
7. Li C, Jiang Z, Yang H. Advances in 3D bioprinting technology for liver regeneration. Hepatobiliary Surg Nutr 2022;11:917-9.
8. Cuvellier M, Ezan F, Oliveira H, et al. 3D culture of HepaRG cells in GelMa and its application to bioprinting of a multicellular hepatic model. Biomaterials 2021;269:120611.
9. Janani G, Priya S, Dey S, et al. Mimicking Native Liver Lobule Microarchitecture In Vitro with Parenchymal and Non-parenchymal Cells Using 3D Bioprinting for Drug Toxicity and Drug Screening Applications. ACS Appl Mater Interfaces 2022;14:10167-86.
10. Ma X, Qu X, Zhu W, et al. Deterministically patterned biomimetic human iPSC-derived hepatic model via rapid 3D bioprinting. Proc Natl Acad Sci U S A 2016;113:2206-11.
11. Hong G, Kim J, Oh H, et al. Production of Multiple Cell-Laden Microtissue Spheroids with a Biomimetic Hepatic-Lobule-Like Structure. Adv Mater 2021;33:e2102624.
12. Fang Y, Ji M, Yang Y, et al. 3D printing of vascularized hepatic tissues with a high cell density and heterogeneous microenvironment. Biofabrication 2023;15:10.1088/1758-

5090/ace5e0.
13. Cuvellier M, Rose S, Ezan F, et al. In vitrolong term differentiation and functionality of three-dimensional bioprinted primary human hepatocytes: application forin vivoengraftment. Biofabrication 2022;14:10.1088/1758-5090/ac7825.
14. Ouyang L, Armstrong JPK, Lin Y, et al. Expanding and optimizing 3D bioprinting capabilities using complementary network bioinks. Sci Adv 2020;6:eabc5529.
15. Stringer M, Zeng Z, Zhang X, et al. Methodologies, technologies, and strategies for acoustic streaming-based acoustofluidics. Appl Phys Rev 2023;10:011315.

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