Editorial Commentary

Innovations in liver organoids: the role of 3D bioprinting in creating functional organoids

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The liver, a pivotal organ in human physiology and the largest exocrine gland, undertakes numerous complex functions essential for maintaining systemic homeostasis (1). It is involved in the metabolism of macronutrients—carbohydrates, proteins, and lipids—the synthesis of bile acids, and the detoxification of xenobiotics. While the liver possesses considerable regenerative capacity, this ability is often compromised by pathological insults such as fibrotic remodeling, viral infections (notably hepatitis B and C), and drug-induced hepatotoxicity. These insults contribute to the progression of chronic liver diseases, including steatohepatitis, cirrhosis, and hepatocellular carcinoma (2). Such conditions constitute a significant public health challenge worldwide. Data from the "Global burden of liver disease: 2023 update" underscore their substantial impact: liver diseases resulted in more than 2 million deaths annually, and hepatocellular carcinoma was responsible for 8.3% of global cancer-related mortality in 2020 (3).

Liver transplantation remains the definitive therapeutic intervention for end-stage hepatic diseases (4). Nevertheless, the persistent scarcity of donor organs and the inadequacies of traditional *in vitro* models in pharmaceutical research and pathophysiological studies necessitate innovative alternatives (5,6). In response, the development of functionally competent liver organoids has emerged as a transformative paradigm. These organoids offer unparalleled opportunities for recapitulating hepatic microphysiology, advancing drug discovery platforms, and exploring strategies in regenerative medicine to reduce reliance on donor liver. The liver demonstrates significant structural and functional heterogeneity, comprising various cellular populations, including hepatocytes, cholangiocytes, and Kupffer cells, within a dynamic stromal microenvironment (7). This intricate niche integrates biochemical gradients, mechanical stimuli, and paracrine signaling, all of which are essential for maintaining organ-specific functions. Conventional two-dimensional (2D) monocellular culture systems, though operationally convenient and adaptable to multiple progenitor sources, fundamentally fail to replicate the physiologically relevant cell-extracellular matrix (ECM) interactions that are crucial for hepatic organoid maturation (8). This limitation arises from the absence of three-dimensional (3D) ECM scaffolds and a deficiency in essential morphogenetic regulators, such as superfamily ligands and chemokine gradients.

Organoids represent a promising model system that bridges the gap between traditional 2D cell culture and in vivo models in mice and humans. These organoids can generate intricate cellular architectures and arrangements through 3D hepatic cell culture. This capability not only significantly enhances the precision and reliability of experimental outcomes but also holds substantial potential for liver regeneration by reconstructing the unique vasculature, thereby enabling the comprehensive reproduction of hepatic functionalities. In 2013, Hans Clevers and his research team successfully developed the first liver organoid using $Lgr5^+$ stem cells derived from injured mice (9). These organoids, which expressed Lgr5

and ductal markers, could be clonally expanded in culture and had the potential to differentiate into functional hepatocytes, thus demonstrating their potential for liver regeneration research and therapeutic applications. However, the initial liver-like organoids exhibited significantly less functionality compared to freshly isolated hepatocytes, and the transplanted organoids only marginally contributed to overall liver function. This underscores the necessity for further optimization of differentiation and transplantation protocols. Subsequently, Yuan *et al.* utilized alginate-encapsulated proliferating human hepatocyte (ProliHHs) liver organoids (eLO) to treat liver failure (10). By providing essential liver functions, eLO treatment notably improved the survival rate of mice with post-hepatectomy liver failure (PHLF) and effectively mitigated symptoms of hyperammonemia and hypoglycemia. Despite their potential, liver organoids encounter several limitations, including an incomplete replication of hepatic complexity, immaturity, and functional deficits. These organoids also lack vascularization, face size constraints, and present challenges in reproducibility and scalability. Furthermore, issues with cell source availability and long-term culture conditions further restrict their application. In this context, 3D bioprinting emerges as a promising technological advancement that can address these limitations by enabling the precise fabrication of complex liver tissues with enhanced functionality and vascularization.

3D bioprinting, employing computer-assisted technology and bioinks composed of natural and synthetic polymers, accurately replicates the construction of extracorporeal biological tissue models. It has become an indispensable tool in tissue engineering and regenerative medicine. This technology enables the layer-by-layer arrangement of biomaterials, biochemicals, and living cells with meticulous spatial control, thereby mimicking the complexities of physiological or pathological states. In our previous research, we utilized a bioink formulation containing 5% gelatin and 2% sodium alginate to encapsulate the HepRG cell line, constructing liver organoids through extrusion-based 3D printing (11). After 7 days of *in vitro* differentiation, these organoids exhibited critical hepatic functions, including albumin secretion, drug metabolism, glycogen storage, and enhanced synthesis of liver-specific proteins. Furthermore, transplantation into the $Fah^{-/-}$ $Rag2^{-/-}$ mouse model of liver injury demonstrated the potential of 3D bioprinting to generate human liver tissues that could serve as alternative donors for liver disease treatment. Another study employed photoresponsive hydrogels and volumetric bioprinting to construct liver models with distinct functions (12). In comparison to extrusion-based printing, liver-derived organoids were successfully produced at high density while maintaining viability and hepatic functions.

While 3D bioprinting has been extensively applied in generating functional liver models, it is imperative to recognize and address its inherent limitations. The cultivation of liver organoids requires a substantial quantity of hepatocytes and associated cell types; however, procuring high-quality cell sources remains a formidable challenge. A study successfully fabricated a 3D-bioprinted liver (3DP-liver) using *in vitro*-expanded primary hepatocytes (eHep cells) and a bioink comprising gelatin, sodium alginate, and liver decellularized matrix (LDCM) (13). The 3DP-liver effectively restored impaired liver functions in mice and significantly enhanced the survival of those with liver injury. In this study, eHep cells, isolated from mouse liver and induced to proliferate using hepatocyte expansion medium (HEM), demonstrated the capacity for continuous passaging. These cells retained their characteristic polygonal epithelial morphology and exhibited glycogen storage and uptake of acetylated low-density lipoprotein, indicating effective preservation of hepatic gene expression and function. The vascularization of liver organoids is crucial for their functionality and viability. However, the highly intricate 3D architecture of liver, encompassing features such as lobules and hepatic sinusoids, presents a significant challenge for bioprinting technology. To ensure the functionality of the organoids, bioprinting methods must accurately replicate these complex structures. A recent study employed the Omnidirectional Printing Embedded Network (OPEN) as an innovative support medium to fabricate mini-livers with venous structures, assessing their hepatic functionality and angiogenic potential (14). The researchers used a biofabrication strategy that combined OPEN with methacrylate gelatin (GelMA), designing the printing path in alignment with segmented liver anatomy, and printed liver organoids with live cells encapsulated in GelMA ink. The results indicated that, compared to traditional two-dimensional or 3D bulk culture methods, primary mouse hepatocytes (PMH) self-assembled into hepatic spheroids *in vitro*, expressed a variety of liver-specific markers, and displayed enhanced liver function and hepatic gene expression. Moreover, unlike liver organoids without veins or those created through conventional grid printing, the implantation of mini-livers featuring endothelial-lined veins facilitated *in vivo* neovascularization.

OPEN, characterized by a polymer network founded on hydrophobic interactions, offers numerous advantages. It is easy to prepare, allows for the direct removal of scaffolding, and is compatible with diverse bioinks. Furthermore, it ensures support stability, precise printing capabilities, and biocompatibility, all of which contribute to high-resolution manufacturing. The liver, with its multifaceted functions, necessitates meticulous regulation of microenvironmental parameters for effective construction. Zhou *et al.* developed the "Chronotoxici-plate", an innovative system employing microfluidic and 3D printing technologies (15). This system consists of a 96-well plate containing circadian-synchronized droplet-engineered primary liver organoids (DPLO) and enables their establishment within 4 days. They utilized cryptochrome 1 (Cry1) as a circadian marker in DPLO and conducted a temporal therapeutic toxicity assessment of oxaliplatin over one week. The study revealed that toxicity of oxaliplatin varied with dosing time. Microfluidic technology advances the construction of liver functions through precise parametric control within the microenvironment, including culture medium flow rates and biomechanical shear stress levels. This approach optimizes the hepatocyte microenvironment, thereby enhancing liver function construction efficacy.

Despite the substantial advancements in 3D printed liver organoids, challenges persist in replicating intricate vascular networks, constructing fully functional hepatic tissues, and addressing the high costs associated with organ printing. Overcoming these obstacles could facilitate the development of transplantable liver tissues, thereby alleviating the shortage of donor organs. Furthermore, obtaining regulatory approval and achieving commercialization of 3D bioprinting are crucial for its expansive application in pharmaceutical and clinical settings.

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