Editorial Commentary

Simulating the *in vivo* tumor microenvironment—advances in building a vascularized model of hepatocellular carcinoma through 3D bioprinting

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Over the past decade, there has been notable progress in the systemic treatment of liver cancer. However, despite the emergence of new therapeutic strategies, they have not universally achieved success, with patients afflicted by liver diseases frequently displaying resistance to these treatments (1). Consequently, liver cancer remains a global health challenge, and hepatocellular carcinoma (HCC) stands as the fourth most common cause of cancer-related deaths globally, constituting 80–90% of primary liver cancer cases (2,3). This poses a substantial threat to both the survival and overall well-being of individuals.

The majority of HCC patients are diagnosed at an advanced stage, and conventional treatment modalities such as surgery and ablation exhibit limited efficacy. The absence of clinically relevant models reflecting the heterogeneity of HCC hampers the development of effective precision therapies for liver cancer (4).

For early-stage liver cancer, surgery or local treatment is the preferred curative therapy. In specific cases, liver transplantation is considered the optimal treatment choice, and trans-arterial chemoembolization (TACE) is a more delicate alternative method (3,5,6). While systemic treatments can provide benefits for advanced HCC, the available options for drugs are significantly limited. Sorafenib, the first approved multi-targeted receptor tyrosine kinase (RTK) inhibitor, has been used as a first-line therapeutic drug for HCC (7). Lenvatinib marks the first successful challenger to sorafenib as the primary systemic therapy for HCC. Mechanistically, as a multi-target RTK inhibitor, lenvatinib effectively inhibits various pro-angiogenic and oncogenic signaling pathway-related RTKs crucial for tumor proliferation. These include platelet-derived growth factor (PDGF) receptors platelet-derived growth factor receptor (PDGFR), Kinase insert domain receptor (KIT), and rearranged during transfection (RET), it also selectively inhibits kinase activity of vascular endothelial growth factor (VEGF) receptors and fibroblast growth factor (FGF) receptors (8). It has been reported that lenvatinib demonstrates non-inferiority in overall survival (OS) (13.6 *vs.* 12.3 months) and demonstrating a higher objective response rate (ORR) according to Response Evaluation Criteria in Solid Tumours (RECIST) (18.8% *vs.* 6.5%), stands as another approved option (9). With the continuous advancement of immunotherapy research, atezolizumab and bevacizumab have become the gold standard in patients not advanced HCC. HCC is characterized by high heterogeneity, easy metastasis and recurrence, and poor prognosis. Herein, precision and personalized medicine research are crucial in the treatment of HCC.

The traditional two-dimensional (2D) cell culture methods based on single-cell layers have been widely used. Despite their simplicity of operation and versatility with various cell sources, they have certain limitations in accurately reflecting the interactions between cells and the tumor microenvironment (TME) due to the lack of extracellular matrix (ECM) as a scaffold, as well as the absence of TME components such as growth factors and cytokines. Patient-derived xenografts (PDXs), which reflect the characteristics of the parent tumor, face challenges such

as species differences in tumor-host interactions, lack of adaptive immunity, ethical concerns, and the substantial human, material, and financial resources required for PDXs construction. Therefore, for patients with a short survival period, PDXs may not be suitable for rapidly assessing drug responses within a limited clinical timeframe (10). Three-dimensional (3D) bioprinting is a method that utilizes computer-assisted technology to more accurately simulate the construction of extracorporeal biological tissue models. In the 3D bioprinting system, living cells are encapsulated in bioinks, which are composed of natural polymers such as alginate, gelatin, collagen, glucosamine, silk fibroin protein, nanoclay and hyaluronic acid. These bioinks are then layered to form a 3D tissue scaffold (11). Compared to traditional methods like 2D flat culture and PDXs models, 3D bioprinting not only more accurately reproduces the characteristics of the parent tumor but also provides increased efficiency and a simpler operational process, rendering it suitable for industrial-scale production. This approach enables rapid and continuous testing and screening of multiple groups, utilizing fewer samples to swiftly assess the impact of various variables on the model. Consequently, it broadens the scope of possibilities for scientific research and medical applications. In order to better simulate the *in vivo* micro-environment, Liu *et al.* used a mixture of 3% gelatin methacrylate (GelMA) and 0.25% fibroin as the material for bioink. The researchers encapsulated human umbilical vein endothelial cells (HUVECs), mesenchymal stem cells (MSCs), and the HepG2 cell line in the hydrogel. They utilized extrusion-based 3D printing to construct a liver vascularization model and measured the expression of genes related to liver function. The study found that the multicellular co-culture model exhibited superior liver functional characteristics compared to the single-cell model. Furthermore, the researchers discovered that the liver vascularization model constructed through 3D printing, when implanted subcutaneously in mice, resulted in the appearance of new blood vessels in the liver tissue on the 7th day post-transplantation. Through arterial vessel transplantation in rats, it was demonstrated that these tissues could be directly used for surgical anastomosis with the host blood vessels (12).

Taymour *et al.* utilized a coaxial extrusion-based 3D bioprinting technique to develop a novel liver sinusoid model. This model comprises a core chamber containing vascular pre-structures and a shell chamber containing liver cells. HepG2 cells are encapsulated within a shell composed of alginate and methylcellulose (algMC), while HUVECs and fibroblasts supporting matrix formation [normal human dermal fibroblast (NHDF)] are encapsulated in a core ink composed of collagen fiber and gelatin. The research results indicate that cell interactions occurring in the triple co-culture model enhance the secretion of albumin (13). This demonstrates that core-shell bioprinting has proven to be a viable model for studying cell-cell interactions.

Wang *et al.* utilized microfluidics to fabricate porous microspheres [poly (lactic-co-glycolic acid) (PLGA) porous microspheres (PMs)] based on PLGA. HepG2 and HUVECs were successfully encapsulated within PLGA PMs, establishing a 3D liver cancer model. The researchers investigated the application of this disease model in drug screening to assess various chemotherapeutic agents, including cell responses to doxorubicin and cisplatin. The study results demonstrated that, under the same culture time, cells under 3D culture conditions exhibited significantly increased drug resistance compared to traditional 2D culture conditions, indicating that cells under 3D culture have enhanced resistance to drugs (14). Constructing 3D-bioprinting vascularized tumor model can be employed in the screening of a broader range of anti-tumor drugs.

Extracting patient-derived tumor cells poses challenges as they are prone to losing their original genetic characteristics in prolonged *in vitro* culture. These cells demand a precise culture environment and experimental procedures, necessitating cultivation in a medium with specific growth factors. These complexities make it challenging to conduct subsequent experiments, thereby limiting the advancement of personalized treatment research. Our research team has dedicated several years to 3D bioprinting personalized therapy, successfully utilizing patient-derived liver cancer cells and colorectal cancer liver metastases cells for *in vitro* 3D bioprinting. This effort has led to the creation of multiple individualized models, employed for personalized screening of diverse chemotherapy and targeted drugs (15,16). The successful establishment of the 3D bioprinting personalized liver cancer drug screening model reflects great potential in the individualized screening of drugs, and may have great commercial application value in large-scale drug testing and personalized drug screening of cancer patients in the future. Furthermore, the construction of multi-cell co-culture models allows for better simulation of the *in vivo* TME. By incorporating patient-derived immune cells [such as macrophages, T cells, B cells, natural killer (NK) cells, etc.], vascular endothelial cells, and fibroblasts, a more intricate and detailed 3D bioprinting model can be constructed. This direction warrants further exploration for achieving high-throughput precision treatment.

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Footnote

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