How to Cite:
Abady, M., Zahran, I. A., & Elmokhtar, Y. (2024). The liver organoid's past, present and future: a personalized medicine strategy. *International Journal of Health Sciences*, *8*(S1), 972–998. https://doi.org/10.53730/ijhs.v8nS1.14980

The liver organoid's past, present and future: a personalized medicine strategy

Mariam M. Abady
Division of Chemical and Matrial Metrology, Korea Research Institute of Standards and Science, Yuseong-gu, Daejeon 34113, Republic of Korea | Department of Bio-Analytical Science, University of Science and Technology, 217 Gajeong-ro, Yuseong gu, Daejeon 34113, Republic of Korea | Department of Nutrition and Food Science, National Research Centre, Dokki, Cairo, 12622, Egypt
Corresponding author email: abadymariam@gmail.com ; mariam.mahmoud@kriss.re.kr (M.M. Abady)

Ibrahim A. Zahran
Chemistry Department, Faculty of Science, Helwan University, Ain Helwan, 11795, Cairo, Egypt

Yasmin Elmokhtar
Physiology Department, Faculty of Dentistry, Pharos University in Alexandria, Alexandria, Egypt

***Abstract*---**Organoids are 3D systems for cell culture made from human pluripotent stem cells or organotypic differentiation with resident cell types. This represents significant multicellular properties, such as interactions and crosstalk between different cell types at the micro- to millimeter-scale, which are morphological and functional markers of actual organs. Such benefits are crucial for studying human tissue and organ biology instead of using animal models, which are constrained by sample accessibility issues and ethical considerations. Due to the challenges in creating human primary liver tissue and patient-specific hepatic disease models, liver organoids are remarkably state-of-the-art. Liver organoids have been developed to study the hepatic phenotype, incorporating different cell types and allowing investigation of cellular, molecular, and genetic aspects of liver diseases, drug metabolism, and protein secretion. They hold promise for fundamental research, drug discovery, and regenerative medicine applications in the study of liver illnesses. Although though organoids have intriguing characteristics, their actual usefulness is constrained by a number of factors, including their relative simplicity, a lack of high-fidelity cell types, flexibility, general arealization applicability of *ex vivo* tissue culture, and their atypical physiology.

Manuscript submitted: 18 May 2024, Manuscript revised: 09 June 2024, Accepted for publication: 27 July 2024

Access to organotypic liver-like surrogates, a range of cell types with *in vivo*-like interactions/architecture, and other disciplines like microfluidic chip technology will dramatically enhance models for illness, toxicity, and drug discovery and pave the way for new treatments. An overview of liver organoids' history and development will be provided, their up-to-date progress, challenges and applications as well as prospects for such technology in the future.

***Keywords*---**Organoids, Stem cell model, liver development, modeling liver disease, personalized medicine.

Introduction

In the human body, the liver serves a number of important functions, including maintaining homeostasis and performing various functions such as water and electrolyte regulation, bile production, detoxification, and metabolism. It plays a crucial role in supporting immunity, digestion, detoxification, metabolism, and vitamin storage. The liver, which makes up around 2% of an adult's body weight, is regarded as a crucial organ for digestion [1, 2]. Its structure consists of hepatic lobules, which are made up of hepatocytes, along with hepatic sinusoidal cells, the biliary system, arterial system, and stroma. Different cell types, including kupffer cells, hepatocytes, liver sinusoidal endothelial cells, and hepatic stellate cells, contribute to the functions of liver [3].

Liver organoids have emerged as a valuable tool for studying liver biology and disease. They are three-dimensional culture systems that closely resemble the organization and function of the liver. Compared to traditional 2D cell cultures, organoids provide a more realistic representation of the organ and allow for the manipulation of signaling pathways and genome editing while maintaining physiological relevance. Liver organoids have shown promise for studying liver development (Fig. 1), disease modeling, drug screening, and personalized medicine [4]. From isolated liver stem/progenitor cells or pluripotent stem cells (PSCs), liver organoids can be produced. During liver organogenesis, the migration of liver cells plays a crucial role. The hepatic endoderm undergoes a transition from an epithelial to a mesenchymal state, leading to the formation of hepatic cords and the development of the liver bud [5]. Hepatoblasts, which are liver progenitor cells, are involved in three-dimensional collective cell movement and morphogenesis [6].

The development of liver organoids involves the coordinated growth of multiple cell types to mimic the complex cellular composition of the liver. Pluripotent stem cells, such as induced pluripotent stem cells (i PSCs) or embryonic stem cells, can be used to initiate liver organoids. Various combinations of cells, including mesenchymal stem cells, hepatic endoderm, and endothelial cells, are employed to generate multilineage liver organoids (Table 1). Future advancements in liver organoid research are required to improve differentiation protocols, achieve better maturation and accurate representation of liver cell types [5]. Increasing the complexity of liver organoids is crucial to accurately mimic pathophysiological conditions in diseases.

Liver organoids hold great potential for personalized medicine and therapeutic applications. They have proven to be effective in modeling liver diseases and providing a progressively realistic choice in personalized liver medicine for disease- and patient-specific treatment plans. Addressing the challenges in liver organoid research will further advance the field and bring tissue engineering closer to reality in the treatment of liver illnesses. As an alternative model system to study the growth and diseases of the human liver, liver organoids are a new emergent technology that we introduce in this review. Additionally, we will talk about the development of liver organoids throughout history, their up-to-date progress, challenges and applications as well as prospects for such technology in the future.

Table 2. Overview of the liver organoid model

Cell source	Cell types	Features	Modeling	Pros	Cons	Developmental stage	References
Biliary Epithelial	Hepatocyte Cholangiocyte	Phase I and Phase II activity Hight CYP3A4 activity	ALGS	Long term maintenance Genetically Stable	Only parenchymal model	Mixed fetal and adult hepatocyte functions	**[7]**
iPSC	Hepatocyte Cholangiocyte Kupffer cell Stellate cell	CYP3A4 expression LPS storage Lipid acculmulation HSC activation Vitamin A storage	NAFLD Fibrosis response Wolmans disease	Multiple cell types Inflammatory response	High inter-batch variability Some HSC activation in routine culture Low Kupffer cell number	Fetal like hepatocyte activity	**[6]**
iPSC HepaRG	Hepatocyte Stellate cell	Retinol storage Drug metabolism ECM production Activatable HSCs	Fibrosis response Toxicity	Enhanced phenotype of both cell types over monoculture	Non-tissue like organization Parenchymal cell lines	Quiescent phenotype and mature HSC Lipid storage Omics difference to primary cells	**[8]**
iPSC	Hepatocyte Cholangiocyte Low abundance of Mesenchymal cell	Bile acid secretion Albumin synthesis CYP metabolism	Biliary disease-ALGS Liver regeneration	Progenitor population Long term maintenance	HSC activation	Liver and PHH transcriptome analysis	**[9]**

Alagille syndrome (ALGS): non-alcoholic fatty liver disease (NAFLD); extracellular matrices (ECM); hepatic stellate cells (HSCs)

Cell Sources for producing Hepatocyte-Like Cells and development of three dimensional liver organoids systems

The choice of cell source for liver transplantation and drug screening is a topic of debate, and various cell types have been explored as alternatives to human hepatocytes. Neonatal hepatocytes have shown better outcomes even after cryopreservation [10]. Endodermal cells, mesenchymal stem cells, embryonic stem cells, induced pluripotent stem cells (iPSCs), and hepatic stem/progenitor cells have been utilized to generate hepatocyte-like cells for preclinical research (Fig. 2). These cells exhibit hepatocyte-like characteristics and offer a solution to the

limited availability of primary hepatocytes. Researchers have successfully generated hepatocyte-like cells using these different cell sources, providing a valuable tool for liver research and drug development. Studies conducted by Xie et al. (2021), Afshari et al. (2020), and Nitta et al. (2020) have demonstrated the generation of hepatocyte-like cells using these different cell sources [3, 11, 12].

Embryonic Stem Cells

Pluripotent embryonic stem cells can differentiate into hepatocyte-like cells, providing a resource for liver research and regenerative medicine. The differentiation process involves multiple stages, starting with the conversion of stem cells into definitive endoderm. Establishing an appropriate culture system is essential for maintaining pluripotency and promoting the differentiation of stem cells into hepatocyte-like cells [13]. Culturing methods can be feeder-dependent while Matrigel, collagen, human recombinant laminin, and its subclass are used in feeder-free cultures or feeder-free, utilizing different substratum materials [14]. Optimizing differentiation protocols and creating the best culture environment can enhance the production of hepatocyte-like cells from pluripotent stem cells, expanding their potential applications. This approach enables the generation of diverse populations of hepatocyte-like cells, contributing to the understanding of liver development, disease modeling, and drug testing. It also offers an alternative cell source to address the limitations of hepatocyte availability for preclinical studies [14]. Continued research and refinement of culture techniques will further advance the field and facilitate the translation of stem cell-derived hepatocytes into clinical applications.

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are a kind of adult multipotent progenitor cells that keep self-renewal capabilities plus can differentiate into various specialized cell types. These cells exhibit specific characteristics, including self-renewal capacity, multipotency with the potential to differentiate into osteogenic, chondrogenic, and adipogenic lineages, and the expression of surface markers such as CD105, CD90, and CD73 and while lacking the expression of CD45, CD34, CD14 and human leukocyte antigens (HLA-DR) [15, 16].

MSCs can be found in different organs, such as adipose tissue, menstrual blood, bone marrow, and the umbilical cord. Among these, MSCs derived from the umbilical cord have garnered significant attention for their potential in treating severe liver diseases and inducing hepatocyte differentiation. Studies have demonstrated the existence of a distinct type of stem cell in the liver that can successfully differentiate into hepatocyte-like cells and shares similarities with MSCs. These liver stem cells show a phenotype akin to mesenchymal stem cells, highlighting their potential part in liver regeneration besides therapeutics [17, 18].

Endodermal Cells

Endodermal stem cells are present in organs derived from the germ layer, such as the liver, as well as organs originating from the endoderm, including the gallbladder, pancreas, and intestine [19]. These cells have the capability of quickly

dividing and differentiating into cells resembling hepatocytes. Endodermal stem cells derived from tissue are a cost-effective alternative to pluripotent stem cells. However, their *in vivo* expansion is limited due to their embryonic developmental stage [3]. In culture, hepatocyte-like cells can be generated by combining mature endodermal progenitor cells obtained from pluripotent stem cells. A study conducted by Sambathkumar et al. in 2018 supports these findings [19].

Hepatic Stem/Progenitor Cells

Two distinct kinds of multipotent cells are present in the liver: hepatic stem cells and hepatoblasts. Hepatoblasts, found in the Hering canals of liver in the adult, are diploid bipotent cells capable of differentiating into hepatocytes and cholangiocytes [20]. Human pluripotent stem cells, which are multipotent hepatoblast progenitors, have potential to generate all types of pancreatic islet cells except for hepatocytes and cholangiocytes [20]. However, obtaining and organizing human pluripotent stem cells and hepatoblasts can be challenging due to their scarcity, representing just 0.5-2.5% of the liver parenchyma from all donors [21].

Hepatic Organoids from Pluripotent Stem Cells

iPSCs have emerged as a promising option for generating liver-like cells due to their ability to maintain the phenotypic traits of hepatocytes, liver sinusoidal endothelial cells, and Kupffer cells. iPSCs offer the advantage of generating various liver cell types, incorporating mesenchymal, hematopoietic, and epithelial cells to mimic the complexity of the liver in vivo. Liver organoids derived from pluripotent stem cells exhibit superior performance compared to those from primary cells, enabling more accurate studies of organ development and diseases [22]. iPSCs, derived from somatic cells, possess a high proliferative capacity and can differentiate into hepatocytes and other cell types, providing a valuable tool for liver research and disease modeling. Continued advancements in iPSC technology hold promise for further enhancing the generation of liver-like cells and their applications in regenerative medicine and drug discovery [23].

iPSC-derived hepatocytes offer a reliable and abundant source for drug screening and liver research. Complex liver organoids, including liver bud-like structures and bile duct architecture, have been successfully generated using iPSCs and pluripotent stem cells (PSCs). However, the full maturation and functionality of iPSC-derived hepatocytes are limited in two-dimensional culture. Nonetheless, iPSCs and PSCs hold promise for studying liver biology, disease modeling, and drug development through liver organoid research. Further advancements in this field are anticipated to enhance the generation and utilization of iPSC-derived liver organoids, allowing for more accurate investigations of cellular interactions and disease mechanisms [22, 24].

IPSC Derived- Hepatocytes like

Hepatocytes, the main functional cells of the liver, perform vital metabolic functions in the body. Liver lobules, organized in a hexagonal structure, consist of hepatocytes and other liver cells, each with specific roles. Different regions of the

liver lobules have specific roles, with mid zone hepatocytes involved in metabolic processes mediated by cytochrome P450, and pericentral hepatocytes responsible for bile acid synthesis and ammonia metabolism [25]. Gene expression and metabolic zonation contribute to hepatocyte specialization. Human pluripotent stem cells can be differentiated into hepatocytes through specific developmental stages, offering a potential source for cell therapy and hepatocyte generation. Established protocols enable the production of large quantities of human pluripotent stem cell-derived hepatocytes, mimicking embryonic development and liver organogenesis [26, 27].

IPSC Derived- Cholangiocytes

Cholangiocytes, specialized epithelial cells in the liver, play a crucial role in bile movement and are involved in cholangiopathies. They maintain bile properties and bile duct structure through primary cilia. Recent research has identified a population of cholangiocytes generated from human pluripotent stem cells, offering potential for studying liver diseases. CFTR-positive cholangiocytes capable of generating functional ciliated cholangiocytes have been identified from human pluripotent stem cells. Differentiation techniques focus on generating hepatoblasts to obtain functional cholangiocytes [28, 29].

IPSC Derived- Non-Parenchymal Cell

Non-parenchymal cells, which include lymphocytes, biliary cells, hepatic stellate cells (HSCs), liver sinusoidal endothelial cells (LSECs), and Kupffer cells, constitute a significant portion of liver cells. These cells contribute to the spontaneous formation of liver tissue and play a vital role in maintaining liver functions through interactions with hepatocytes. Hepatic stellate cells, in particular, are involved in vitamin A storage and liver expansion [30, 31]. Liver endothelial cells are responsible for maintaining the liver's stiffness through the production of extracellular matrix components. However, the interactions between different liver cell types are not fully replicated in traditional differentiation systems due to the lack of non-parenchymal cells [31]. Pluripotent stem cells (PSCs) offer the potential to generate various hepatocytes, cholangiocytes, and other non-parenchymal cells, which self-organize and contribute to the structural components of the liver. This allows for the generation of liver-like structures that closely mimic the complex structure and functionality of the liver [32].

Organoids derived from Adult stem cells

Adult stem cells, sometimes referred to as undifferentiated committed lineage cells, have the potential to be extremely important in liver regeneration. Significant liver damage may prompt differentiated cholangiocytes or hepatocytes to reenter the cell cycle, resulting in the generation of adult stem cells and promoting liver healing. Adult stem cells can only develop into specific sections of the organ or tissue from which they originate, in contrast to organoids made from PSCs [33]. Adult stem cell organoids exhibit chromosomal and structural stability, allowing them to regulate the frequency of genetic alterations and maintain functional integrity. These organoids lack the genetic and epigenetic abnormalities often observed in iPSCs -derived organoids [34]. Adult stem cells

are found in unique microenvironments known as stem cell niches, which can modify tissue homeostasis and affect stem cell behavior. Adult stem cells have extraordinary plasticity in how they can be controlled by mechanical stimulation and communication within the stem cell niche [35, 36].

Applications of liver organoids

Superior to typical tissue culture techniques, organoids offer numerous great advantages. They can simulate 3D tissue structure and function as well as disease pathology in a near-physiological manner. Consequently, they have shown enormous ability to become superb platforms for a variety of applications in fundamental and translational research (Fig. 3) [37].

Liver organoids as disease models

Liver organoids can be generated from diseased tissues driven from a patient as well as from healthy tissues, which is what makes these organoids employed for disease modelling. Patient-originated organoids maintain an individual's genetic background, with the disease-causing mutations, enabling them to be used in personalised medicine and therapeutics efficacy studies. Furthermore, via gene editing, pathological genetic mutations can be introduced into healthy organoids to examine their contribution in pathogenesis and responsiveness to therapies. The next sections explain the developments of organoid culturing techniques in modelling of liver diseases (Fig. 4, Table 1) [48].

Table 1. Liver organoids application as disease modelling

Disease	Species	Source	Reference
Alagille syndrome	Human	Biopsy	[38]
		iPSCs	[39]
	Mouse	Adult tissue	[40]
Alpha-1 antitrypsin deficiency	Human	Biopsy	[38]
Cystic fibrosis	Human	iPSCs	[29]
HBV infection	Human	iPSCs	[41]
HCV infection	Human	iPSCs	[42]
Liver cancer	Human	Biopsy	[42]
	Human	Adult tissue (GE)	[43]

ESCs, embryonic stem cells; GE, genetically engineered

Genetic diseases

Alagille syndrome (ALGS) is a genetic disorder primarily caused by mutations in the JAG1 gene, leading to impaired bile duct development and chronic cholestasis. Liver organoids derived from ALGS patients [40], mice models [44], and iPSCs have provided insights into the disease pathology. Using CRISPR/Cas9 technology, disease-specific organoids with ALGS-like features can be generated from healthy iPSCs by introducing the disease-causing mutation [39]. ALGS organoids exhibit underdeveloped cholangiocytes and bile duct structures, which can be restored by reversing the mutation. These ALGS organoids serve as

valuable models for studying disease mechanisms and developing potential therapies for ALGS [39].

α1-antitrypsin deficiency (A1AT) is a genetic disorder caused by a mutation in the SERPINA1 gene, resulting in reduced production of α1-antitrypsin, a protein that protects the lungs [45]. Liver organoids derived from A1AT patient biopsies were generated and exhibited protein aggregates within the cells, along with decreased secretion of α1-antitrypsin. This study provides insights into the cellular manifestations of A1AT deficiency and highlights the potential of liver organoids for modeling and studying the disease [38].

Citrullinemia type 1 (CTLN1) is a rare metabolic disorder caused by mutations in the arginosuccinate synthetase 1 (ASS1) gene, leading to hyperammonemia [46]. Liver organoids derived from patient biopsies were used to model CTLN1, revealing the absence of ASS1 expression. In contrast, healthy organoids exhibited normal ASS1 expression. By reversing the ammonia accumulation associated with the disease using genetic manipulation techniques, ASS1 expression was restored in CTLN1 organoids. This study highlights the importance of ASS1 abnormalities in CTLN1 and demonstrates the potential of liver organoids for disease modeling and therapeutic development [38].

Alcohol-related and non-alcoholic fatty liver diseases

Alcoholic liver disease (ALD) is closely connected to chronic alcohol consumption and is influenced by genetic factors (e.g., TM6SF2 and PNPLA3), obesity, malnutrition, and viral infections. Liver organoids co-cultured with human fetal hepatic mesenchymal cells can replicate the pathophysiology and progression of ALD, including liver fibrosis induced by oxidative stress and inflammation. This disease model provides a platform to study steatohepatitis, a condition that occurs in ALD [47].

Non-alcoholic fatty liver disease (NAFLD) is influenced by various factors such as sedentary lifestyle, high-energy diet, obesity, diabetes, and hyperlipidemia [48]. Hepatocytes exposed to free fatty acids develop steatosis, ballooning, and increased pro-inflammatory cytokines and collagen, mimicking the features of non-alcoholic steatohepatitis (NASH) where inflammation and fibrosis play a significant role [49]. Patient-derived organoids are valuable for modeling specific diseases, and NASH organoids exhibit distinct transcriptomes and activities, including increased growth, lipid accumulation, and susceptibility to apoptosis. Co-culturing NASH organoids with stellate cells, Kupffer cells, and T cells effectively replicates NASH-associated inflammation, fibrosis, and tumorigenesis. This approach more accurately reflects the liver microenvironment of NASH patients compared to models generated from healthy organoids [50].

hPSCs have shown promise in modeling non-alcoholic fatty liver disease (NAFLD) and exploring its underlying molecular mechanisms. In a study, hPSCs were differentiated into hepatocyte-like cells (HLCs) and cultured as three-dimensional organoids to mimic NAFLD features. These organoids exhibited lipid accumulation and upregulated gene expression related to lipid metabolism and inflammation, reflecting the characteristics of NAFLD. The researchers also observed the

overexpression of a specific gene, PLIN2 (perilipin 2), known to be involved in lipid storage and associated with NAFLD progression. Additionally, the study investigated the role of peroxisome proliferator-activated receptor alpha (PPARα), a key transcription factor in lipid metabolism. Activation of PPARα in the organoids resulted in reduced lipid accumulation and downregulation of PLIN2 expression, suggesting its potential therapeutic role in NAFLD. The findings highlight the ability of liver organoids derived from hPSCs to recapitulate NAFLD features and provide a valuable platform for studying the disease mechanisms and assessing potential therapeutic interventions. Liver organoids offer a more physiologically relevant model compared to traditional cell culture systems, enabling researchers to better understand the complex interactions among different cell types and explore personalized treatment strategies for NAFLD. The overexpression of specific genes in NASH organoids holds promise for future applications in NASH diagnosis and treatment [51-53].

Liver fibrosis

Liver fibrosis is a pathological condition characterized by the abnormal accumulation of connective tissue in the liver due to chronic inflammation [54]. This condition arises from various factors such as fatty liver disease, viral hepatitis, immunological disorders, or exposure to certain chemicals or medications. In liver fibrosis, hepatic perisinusoidal stellate cells are stimulated by inflammation, leading to their activation and differentiation into myofibroblasts. These myofibroblasts secrete excessive amounts of extracellular matrix, contributing to the fibrotic process [54]. The chronic inflammatory response also triggers the activation of immune cells, which further damages liver cells and impairs their ability to regenerate effectively [55].

HepaRG-based 3D liver organoids were co-cultured with stellate cells to create a fibrosis model. Exposure to allyl alcohol and methotrexate induced increased stellate cell activity, collagen secretion, and deposition, mimicking fibrosis development. Additionally, acetaminophen (APAP) administration triggered hepatocyte injury, activating stellate cells and promoting collagen synthesis, while histone deacetylase inhibition prevented their activation [56]. Congenital hepatic fibrosis, observed in autosomal polycystic kidney disease (ARPKD), was modeled using liver organoids with an ARPKD-specific mutation. The overexpression of transforming growth factor (TGF) induced collagen fiber production in bile duct cells by myofibroblasts, leading to liver fibrosis. These findings highlight the potential of liver organoids in studying fibrosis mechanisms and testing therapeutic interventions [9].

Researchers developed a micropatterned agarose scaffold for high-throughput production of liver organoids. The scaffold consisted of hexagonal microcavity arrays, allowing precise cell placement and reliable organoid formation. The liver organoids exhibited liver-specific gene and protein expression, indicating their hepatic fate. They displayed a cytoarchitecture resembling fetal liver, with a stem cell niche surrounded by hepatic and non-parenchymal cell areas [57, 58]. The organoids demonstrated liver-specific activities such as albumin secretion, urea generation, glycogen synthesis, and lipid droplet formation. They proved useful for evaluating drug-induced hepatotoxicity and liver fibrotic models, showing

activation of relevant genes and the formation of cystic lumens [9]. However, compared to mature liver tissue, these organoids are still in early stages of maturation, with lower maturation rates and liver function. They also lack the precise cytoarchitecture of mature liver tissue. Overcoming these limitations may be possible by incorporating dynamic and co-culture strategies. Overall, the micropatterned agarose scaffold provides a valuable platform for generating liver organoids and studying liver-related diseases and drug responses [57].

Liver cancer

Primary liver cancer (PLC) is a major cause of mortality worldwide, comprising different subtypes such as cholangiocarcinoma (CC), hepatocellular carcinoma (HCC), and combined hepatocellular-cholangiocarcinoma (HCC/CC) [59]. Traditional research methods using 2D cell cultures and mouse models have limitations in accurately representing the heterogeneity of PLCs [45]. Patient-derived xenografts have been used to mimic tumor characteristics, but they are costly and time-consuming. Liver cancer organoids have been developed using surgically resected tumor tissues from patients, and when transplanted into mice, these tumoroids retained the genetic characteristics and metastatic abilities of the original tumors [60]. Needle biopsies have also been utilized to generate organoids of HCC and CC. However, the generation of tumoroids is more successful for poorly differentiated and highly proliferative cancers, limiting their use in early-stage cancers. To overcome the limited complexity of organoids, researchers are exploring the combination of cancer cells with stromal and immune cells [45].

Prime editing of healthy induced pluripotent stem cells (iPSCs) or adult stem cells offers an alternative method for creating tumoroids. By manipulating the function of specific genes (BAP1 with cholangiocarcinoma mutations (SMAD4, NF1, PTEN, and TP53) using CRISPR/Cas9 technology), researchers have successfully transformed normal liver organoids into malignant tumoroids. However, the in vitro lifespan of tumoroids is shorter than that of healthy organoids, which restricts their long-term culture applications. In one study, researchers investigated the role of transfer RNAs (tRNAs) in HCC development and their potential as therapeutic targets [61]. They found that several tRNAs were down-regulated in HCC tumors compared to healthy liver tissues. Higher expression of tRNA-Lys-CUU was associated with worse clinical outcomes in HCC patients. Deprivation of lysine, which affects tRNA-Lys-CUU, led to reduced colony formation, migration, cell cycle arrest, and apoptosis in HCC cells. The study also examined aminoacyl-tRNA synthetases (ARSs) responsible for charging tRNAs and observed upregulation of KARS, an ARS involved in charging tRNA-Lys-CUU, in HCC tissues [62]. Knockdown of KARS reduced HCC cell proliferation and induced cell cycle arrest and apoptosis. Additionally, a KARS inhibitor called cladosporin successfully suppressed the proliferation of HCC cells [63]. These findings suggest that amino acid deprivation and targeting ARSs, particularly KARS, could be potential therapeutic strategies for HCC. However, further research is needed to evaluate the efficacy and potential side effects of targeting ARSs in cancer treatment.

Host–microbiome interactions

Hepatitis C virus (HCV) entrance and localisation in hepatoma tumoroids have been studied. HCV accumulates in the basolateral membrane, as well as its virions concentrate at tight junctions over time. This model provides an encouraging method for researching the complex HCV trafficking [43]. Functioning human iPSC-originated hepatic organoids have recently established and infected with the genome of hepatitis B virus (HBV). In comparison to 2D human iPSC-derived hepatic-like cells, these functioning hepatic organoids showed higher vulnerability to HBV infection and could sustain HBV propagation and create infectious virus for longer periods of time. Organoids infected with HBV exhibited unusually significant liver dysfunction, as evidenced via increased liver damage biomarkers and a changed liver ultrastructure. Curiously, it was revealed that hepatic organoids infected with HBV and created without immunological cells exhibited reduced liver function, raising the possibility that HBV is a cytopathogenic virus [41].

Omic profiling

Rather than screening existing drug libraries, omics data could be utilised in order to suggest new therapeutics for diseases. Organoid technology might also be used to amplify adequate amounts of tissues *in vitro*, healthy as well as diseased, to evaluate causative mutations via deep sequencing, and to evaluate therapy routines through characterization of organoids phenotype via omics data platforms. For liver cancer patients, novel therapeutic targets have been recently identified by examining tumoroids' omic profiles and it has been demonstrated that ERK inhibitors can reduce tumour development in patient-originated xenografts [64].

Hepatotoxicity

Hepatotoxicity, the toxic effect of drugs and their metabolites on the liver, is commonly evaluated using 2D primary hepatocytes. However, their reduced cytochrome P450 (CYP) activity limits their long-term functionality. Hepatic organoids, with functional CYP enzymes, offer a promising model for assessing chemical-induced liver damage and drug toxicity [37]. Hepatic organoids express phase 1 and phase 2 enzymes, including cytochrome P450 (CYP450). Omeprazole treatment enhanced the expression of CYP3A4 and CYP1A2 in liver organoids. Compared to 2D hepatocytes, organoids were more sensitive in assessing the toxicity of hepatotoxic drugs mediated by CYP3A4 and CYP1A2/2E1. Organoids showed higher sensitivity to clinically relevant doses of hepatotoxic agents, with approximately 50-fold lower toxic dosage of trovafloxacin in 2D hepatocytes [65]. Finally, Hepatotoxicity analysis using organoids is valuable for drug screening and early detection of liver damage [37]. Organoid models have been developed to assess drug toxicity, liver injury, and mitochondrial toxicity. These models accurately evaluated the effects of numerous drugs (commercially available drugs) [66] and studied hepatotoxicity and lipid metabolism modifications by 1 m PS-MP microbeads of liver organoids derived from hPSCs [67]. Organoid-based toxicity screening provides a new approach for understanding organ toxicology and holds promise for mechanistic research, precision medicine, and drug screening.

Biobanking

Cryopreservation of healthy and diseased human organoids as biobanks will also serve as a renewable supply for the organoid models [68]. These "Living Biobanks" are gaining popularity among academic and industrial researchers for a variety of purposes associated, especially, with the establishment of novel treatment methods, the discovery of innovative diagnostic biomarkers, and the establishment of personalized treatment regimens. The Human Cancer Models Initiative (HCMI) was recently launched with the main target of supplying the scientific world with a vast collection of easily available cancer models, which can be employed to conduct fundamental and translational research in cancer. These might be facilitating the identification of novel pharmacological targets, the creation of fresh diagnostic markers, and the development of preventative and therapeutic strategies for individualized medicine [37].

Transplantation

The ability to produce functional organoids from isogenic self-tissues makes them a hopeful alternative to cell and whole organ transplantation. With iPSC technology, easily available tissue biopsies can also be used to create HLA-matched tissue-specific organoids [69]. Functional engraftment of orthotopically implanted organoids in the kidney [70], liver [37, 71], and brain [72] has demonstrated their ability to repair injured or diseased tissues *in vivo*. The researchers aimed to reprogram human fibroblasts into functional cholangiocyte-like cells, which are specialized cells in the bile ducts of the liver. They used a combination of transcription factors to induce the fibroblasts to acquire cholangiocyte characteristics. The reprogrammed cells, known as induced cholangiocyte-like cells (iCLCs), exhibited similar gene expression patterns and functional properties to native cholangiocytes. The researchers further demonstrated the potential of iCLCs in regenerative medicine by transplanting them into mice with a genetic liver disease. The transplanted iCLCs integrated into the liver tissue and improved the liver function in the diseased mice [73].
The only viable treatment for late-stage liver failure now is orthotopic liver transplantation, but this option is constrained by a dearth of suitable and healthy donors as well as the possibility of immunological rejection. Liver organoids, which have complex structures and metabolic processes, have the ability to grow and differentiate, making them a possible alternative cell source for transplantation (Fig. 5). Numerous hepatic organoid models have been demonstrated to successfully engraft into recipient mice's livers and replenish damaged livers [69].

Collaborative and future applications of liver organoids
Personalized medicine

As mentioned above, liver organoids are essential for personalized medicine, enabling the study of patient-specific liver diseases and tailored treatments. By using iPSCs from a patient to create liver organoids, researchers can replicate individual liver tissue, study disease mechanisms, identify biomarkers, and test drug efficacy [64, 74]. This advancement improves regenerative medicine, personalized medicine and patient outcomes [75, 76].

CRISPR-Cas9 technology and gene therapy

Organoids derived from patients and organoids edited using CRISPR/Cas9 technology are valuable tools for disease modeling and personalized medicine [46]. CRISPR-edited organoids offer advantages by allowing the introduction of specific mutations into healthy organoids, enabling the study of disease mechanisms and therapeutic responses via knockout and knockin systems [61, 77]. Continuous monitoring and intervention at different disease stages are made possible through CRISPR-mediated alterations. Additionally, CRISPR-based genome-scale screening has led to the discovery of new cancer-related genes. Liver tumor organoids created using CRISPR/Cas9 constructs in mice closely resemble in vivo tumors in terms of morphology and protein expression, and they exhibit resistance to radiation. The integration of cutting-edge genome editing techniques with organoid technology shows promise for correcting genetic abnormalities in patient-derived organoids and potentially treating currently untreatable diseases. Recent advancements have demonstrated successful reversal of disease-causing mutations and restoration of disease phenotypes in patient-derived organoids. These developments pave the way for novel therapeutic approaches and improved understanding of disease mechanisms [78, 79]. Notably, recent advancements have shown the successful reversal of disease-causing mutations and restoration of disease phenotypes in patient-originated organoids, such as in ALGS [39].

Researchers evaluated the radiosensitivity of liver cancers with different genetic alterations by inducing mouse liver tumors in vivo using CRISPR/Cas9 constructs. Targeting genes such as Tp53, Pten, Nf1, Nf2, Tsc2, Cdkn2a, and Rb1, they generated tumor organoids from these tumors. The tumor organoids closely resembled the original mouse liver tumors in morphology and protein expression. Notably, tumor organoids with mutant Nf2 displayed enhanced resistance to high-dose radiation compared to organoids with other gene alterations, as observed through an ATP cell viability assay (Fig. 6). These findings highlight the potential of using tumor organoids to study the response of liver cancers to radiation therapy [80].

Bioengineered organoid models

The scientific community has begun to employ numerous bioengineering techniques for controlling the cellular microenvironment such as microfluidics, bioprinting and 3D biomimetic scaffolds in order to enhance the *in vitro* durability and reproducibility of cell functions [81, 82]. In a cell culture apparatus based on microfluidics with a constant flow of fluids, a decellularized 3D liver extracellular matrix hydrogel was used for co-culturing induced hepatocytes (iHeps) directly differentiated from fibroblasts with endothelial cells, thereby developing an iHep-derived vascularized 3D liver organoid model. Using this system, iHep-derived 3D liver organoids could construct a multi-organ system consisting of numerous organoids originated from various internal organs and show significant practicality for standardised and functional high-throughput drug screening [83].
By precisely controlling critical physicochemical parameters such as the flow of fluids and biochemical signals, micro-scale technologies may be able to simulate *in vivo* cellular microenvironments [84, 85]. Through a simple and effective technique, differentiation *in situ*, enduring 3D culture, and the creation of hiPSC-

derived functioning organoids of liver were promoted in a perfusable micropillar chip system. The organoids of liver showed significant development and heterogeneity features including hepatocyte and cholangiocyte differentiation, thereby mimicking the hapatic tissue *in vivo*. The cell survival of liver organoids was improved in perfused culture conditions in addition to expression of endoderm- and mature hepatocyte-specific genes. Furthermore, they showed improved metabolic abilities as well as liver-specific activities such as albumin and urea synthesis. Eventually, the liver organoids displayed hepatotoxic response toward acetaminophen that is dependent of dosage and time, indicating that they are an excellent platform for drug testing [86].

Organ-on-a-chip

Organ-on-a-chip systems combine organoids with microfluidics to create biomimetic 3D hepatic tumor models, enabling drug research and modeling of human cancer microenvironments [87, 88]. Efforts are also being made to develop multi-organ systems to investigate organ-to-organ crosstalk. Integrating organoids and organ-on-a-chip models offers the potential to achieve cellular maturity that may not be possible with either approach alone [89, 90]. Furthermore, the application of mass spectrometry in organoid and organ-on-a-chip technologies extends beyond fundamental research, encompassing areas such as sports doping analysis and environmental toxicology [91-93]. Additionally, mass spectrometry has significant advances for the understanding of organoids and organ-on-a-chip technologies [94]. Interestingly, Wand et al. utilized a perfusable microcapillary chip to develop hepatic organoids capable of measuring the dose-dependent and time-dependent effects [69]. This organ-on-a-chip system showed promise as a novel and operative platform for drug challenging. Another study looked into co-culturing cancer-associated fibroblasts (CAFs) and patient-derived organoids (PDOs) to develop a model that had higher resistance to anti-cancer drugs and mimicked the pathology seen in patients with liver cancer [95]. Kinome profiling, a high-throughput screening method for kinases, was employed to generate organoids of cholangiocarcinoma, which identified possible therapeutic targets and offered therapeutic stratification [96]. These results demonstrate the importance of PDOs and genome-edited hepatic organoids as useful tools for examining the pathways underlying the beginning of liver cancer and creating potential treatments for human liver cancer.

Challenges and future perspective

Liver organoids advance biomedicine by enabling disease modeling, precision medicine, regenerative approaches, and pharmaceutical research. Challenges remain in achieving physiological function, cell diversity, and maturation, requiring further investigation for optimal utilization (Fig. 7) [97-101].

Liver organoids face challenges in replicating native organ structure and cell composition. Single-cell sequencing technology helps compare transcriptional patterns of organoids and target organs [102], showing liver organoids resemble the liver and mimic developmental processes accurately. RNA tomography provides spatially resolved cell transcriptional profiles [103], enhancing organoid characterization and reproducibility for studying human liver development.

Liver organoids derived from PSCs have limited cell maturation, posing a significant drawback. Challenges include the availability of patient-derived tissue, limited hepatocyte yield, and high cost of cell amplification [104]. Standardized protocols for growing liver organoids are lacking, and the use of fetal tissues instead of adult tissues is common. *In vitro* cultures lack *in vivo* environmental elements, further complicating organoid development. Bioreactors offer a solution by enabling continuous culture spinning, nutrient supply, and medium control. Bioreactors extend organoid lifespan, produce more physiologically relevant structures, and hold promise for regenerative medicine applications in liver tissue engineering [105, 106].

Future advancements in organoids involve integrating blood vessel organoids with liver organoids to study hepatovascular interactions. Transplanting liver organoids into mice offers an alternative to long-term culturing. Vascularization techniques, such as sacrificial molds and bioprinting, enable the development of perfusable vascular units within organoid scaffolds. Co-culturing endothelial cells with other cell types promotes vascularization and the formation of self-organizing liver buds with microvascular networks. However, achieving full maturity in patient-specific organoids may require novel approaches to accelerate maturation and model age-related traits. These advancements have the potential to enhance the functionality and lifespan of liver organoids, bringing us closer to mimicking the complexity of the human liver [107].

To improve the mimicry of *in vivo* organs, stem cell culture scaffolds with specific topographies can enhance organoid design and size [108]. Bioengineering techniques enable control over morphogenesis and self-organization, improving organoid topology and stem cell interactions [109]. Microfluidic organ-on-a-chip technology combines key elements of liver organoids, offering a modifiable and reproducible platform. Although high-throughput screening is challenging, mini-liver organoids have been successfully analyzed using automated robotic pipelines. Advancements in robotics and automated phenotyping will further enhance liver organoid technology for high-throughput applications [110]. Combining organoid cultures with organ-on-chip technologies can replicate inter-organ communication. Liver-on-a-chip models enclosed in hydrogels have shown enhanced liver function and personalized drug screening capabilities. However, organoid-based models for studying liver injury are still limited compared to animal and 2D cell models. Organoids-on-a-chip technology has the potential to develop an *in vitro* model of liver ischemia-reperfusion injury (IRI), a significant type of liver damage [102, 111, 112]. Further research is needed in this area for establishing an organoid-based liver IRI model using organoids-on-a-chip technology, which holds promising applications for future studies [113, 114].

Liver organoid culture methods often rely on hydrogel matrices like Matrigel, but these matrices have limitations such as poor definition, complexity, heterogeneity, and inclusion of animal products [115]. Synthetic and chemically specified hydrogels offer a solution by allowing tailoring and optimization for specific applications. Chemically defined hydrogels like polyisocyanopeptides (PIC) and laminin-111 have been introduced for human liver organoid culture, showing promise for therapeutic use [116]. Controlled stiffness nanofibrillar hydrogels have also been developed to mimic real organs [86, 117-119]. However, liver

organoids still face challenges in replicating dynamic cell-cell interactions and encompassing all liver tissue cell types. Further research is needed to standardize and improve liver organoids for broader clinical and laboratory applications [107]. Organoids face challenges in modeling multi-organ diseases, limiting their applications. Co-culturing strategies, such as iPSC-derived liver organoid co-cultures, show promise in studying liver cancer. Advances in iPSC culture techniques enable more sophisticated organoid systems, including vascularized liver organoids [120-122]. Liver co-cultures provide opportunities for studying host-pathogen interactions, like interactions between liver organoids and HBV/HCV [23, 123]. Additionally, co-culturing organoids with immune cells allows for investigating immune responses and evasion mechanisms, which is important in the context of immune-checkpoint inhibitors in treating HCC.

Conclusion

Liver organoids derived from pluripotent stem cells (PSCs) offer a unique platform for studying human liver development and diseases. They can replicate both healthy and pathological features of the liver, providing insights into human-specific aspects of liver biology. These organoids have advantages over animal models and can overcome the limitations of tissue availability. They hold promise in various applications such as drug discovery, personalized medicine, gene therapy, and regenerative medicine. However, there are challenges to be addressed for the practical use of organoids in preclinical and clinical settings. Organoid models have limitations in studying specific cell types, functional circuits, and complex interactions between liver cells. Genetic variations and differences among stem cell lines can also affect interpretation of results. Overcoming these challenges requires a multidisciplinary approach, involving bioengineering and validation through replication in different cell lines. Additionally, controlling self-organization, achieving physiological shapes and sizes, extending organoid lifespan, and incorporating additional tissue compartments are areas of focus. It is crucial to exercise caution when extrapolating in vitro model results to human biology without validation in primary tissue or in vivo models. While organoids offer immense potential, it is important to recognize their limitations and not consider them as a perfect model system. Continued research and advancements in the field are expected to improve the utility and applicability of liver organoids in liver research and therapeutic development.

Authors' Contributions

MMA designed the review paper structure and layout. MMA, IZ, and YE contributed to the preparation of the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ORCID Id

Mariam M. Abady https://orcid.org/my-orcid?orcid=0000-0003-0011-8730

Fig. legends

Fig. 1. History of liver organoid research. The foundation of organoid technology can be traced back to early studies on the re-aggregation of sponge cells, which suggested the importance of self-organization in organ formation. The establishment of pluripotent stem cell lines, including embryonic stem cells and induced pluripotent cells, further advanced the field. Utilizing these stem cell lines, researchers have successfully developed various types of organoids including liver organoids, enabling the modeling of complex tissues. Liver organoid models, in particular, have been extensively employed to investigate human liver disorders. These models have provided valuable insights into the understanding of liver development and disease mechanisms. To overcome the limitations of organoids, such as oxygen deprivation during long-term culture, bioengineering technologies are being developed and implemented.

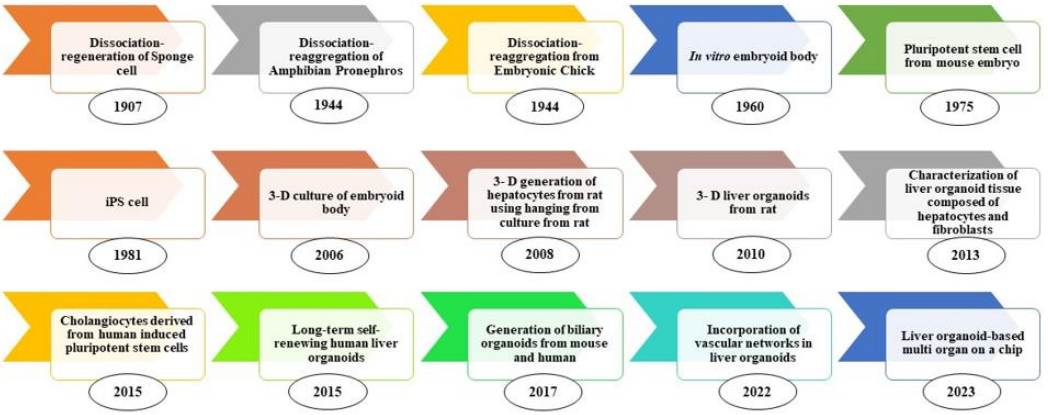


Fig. 2. Diagrams of typical cell type's show primary cells produced from tissues, immortalized cell lines, and cells developed from expandable stem cells to produce liver organoid models for use in personalized medicine.

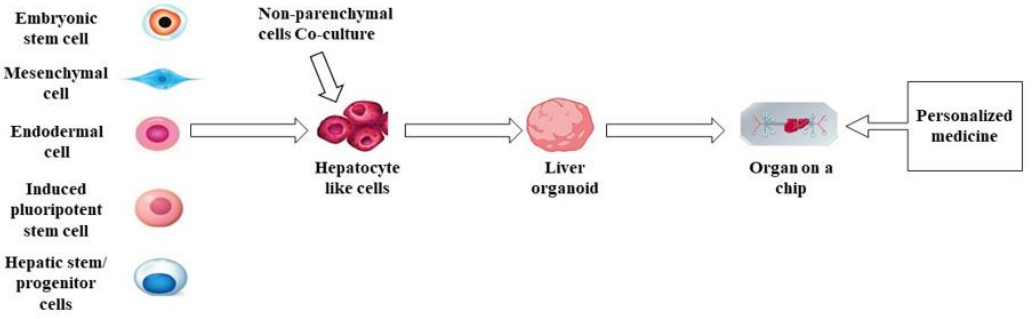


Fig. 3. Applications of liver organoids. Healthy and diseased patient-originated organoids represent a precious model to investigate the physiopathology of the liver. Created with BioRender.com.

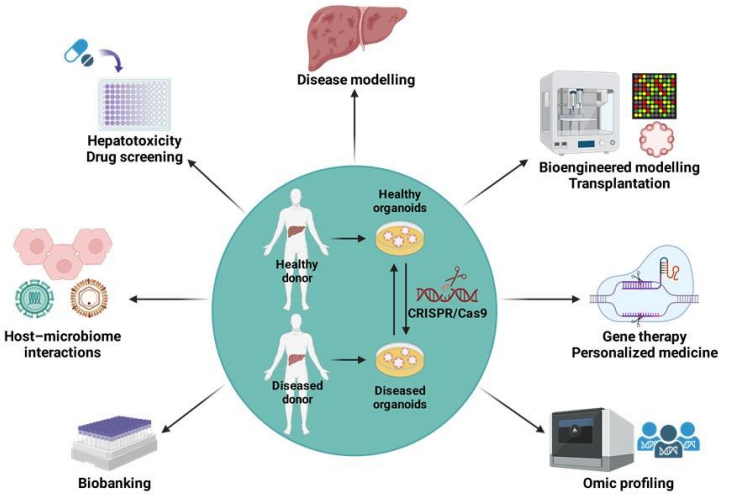


Fig. 4. Liver Organoids for disease modelling. Created with BioRender.com.

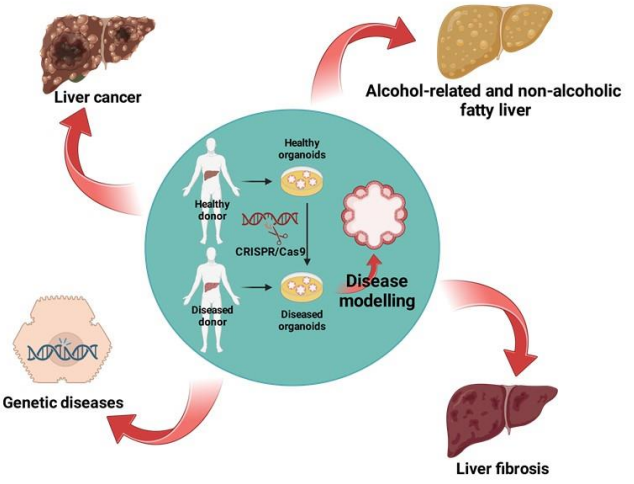


Fig. 5. A diagram showing CRISPR-Cas9 gene-editing technology is used to rescue disease-relevant liver organoids, and then gene-edited organoids are autologously transplanted into people with metabolic disorders. (b) Immune rejection of transplanted allogeneic organoids can be minimized by creating induced pluripotent stem cells (iPSCs) that match the patient's HLA.

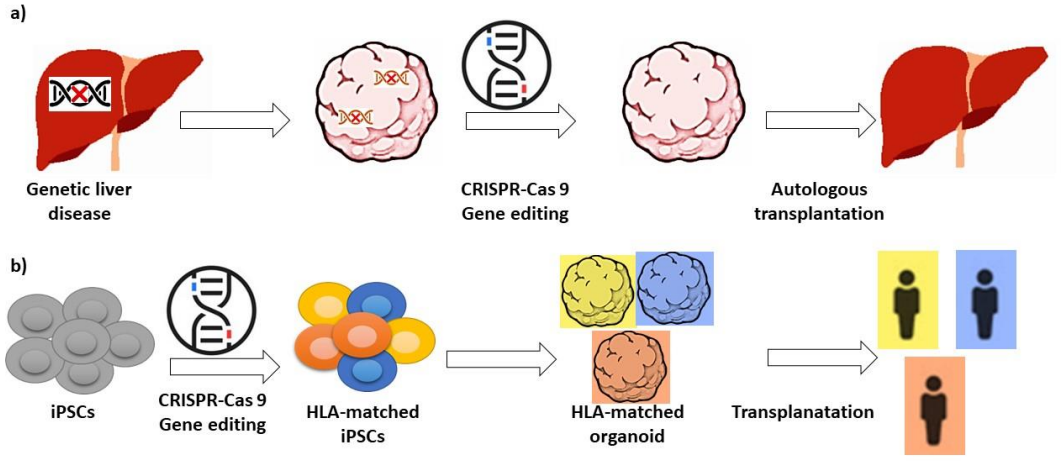


Fig. 6. A diagram showing organoids from the mouse liver tumor were created after the clustered regularly interspaced short palindromic repeat-caspase 9 (CRISPR-Cas9) constructs were hydrodynamically injected into the tail vein of the mouse to create the tumor model. These organoids were then tested for radiation sensitivity.

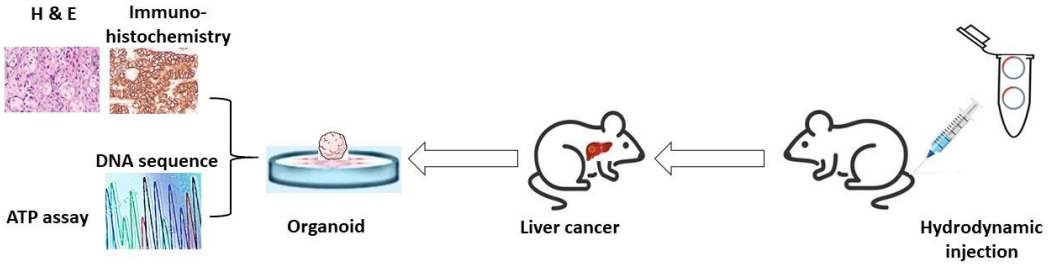
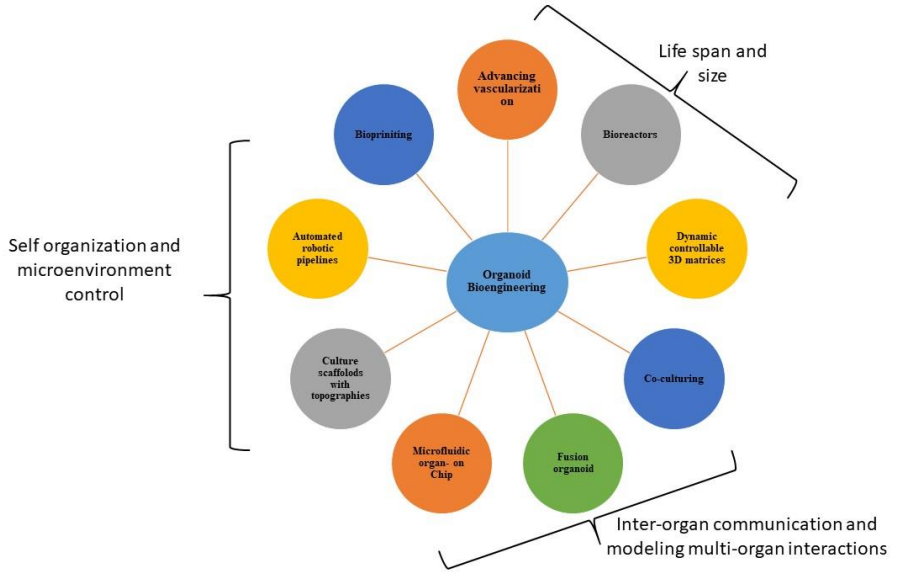


Fig. 7. The next generation of liver organoids aims to overcome current limitations by utilizing bioreactors to enhance nutrient availability and address size constraints. The extracellular environment can be manipulated to guide organoid growth and self-organization towards desired architectures, aided by synthetic materials with adjustable properties. Microstructured cell culture scaffolds offer a means to mimic the topography of target tissues. Integration of organ-on-chip technology enables connectivity and communication between organoids. Co-culturing multiple cell types within organoids facilitates the formation and potential self-organization of diverse tissues. Incorporating vascular networks in organoid culture enhances nutrient availability, approaching physiological conditions. These advancements hold promise for applications in tissue engineering and regenerative medicine, driving the development of more sophisticated and functional liver organoid models.



References

1. Ober, E.A. and F.P. Lemaigre, *Development of the liver: Insights into organ and tissue morphogenesis.* Journal of hepatology, 2018. **68**(5): p. 1049-1062.
2. Chi, K.Y. and J.-H. Kim, *Recent advances in liver organoids and their use in in vitro modeling of non-alcoholic fatty liver disease.* Organoid, 2022. **2**.
3. Xie, Y., et al., *Induction and maturation of hepatocyte-like cells in vitro: Focus on technological advances and challenges.* Frontiers in Cell and Developmental Biology, 2021. **9**: p. 765980.
4. Prior, N., P. Inacio, and M. Huch, *Liver organoids: from basic research to therapeutic applications.* Gut, 2019. **68**(12): p. 2228-2237.
5. Takebe, T., et al., *Massive and reproducible production of liver buds entirely from human pluripotent stem cells.* Cell reports, 2017. **21**(10): p. 2661-2670.
6. Ouchi, R. and H. Koike, *Modeling human liver organ development and diseases with pluripotent stem cell-derived organoids.* Frontiers in Cell and Developmental Biology, 2023. **11**.
7. Huch, M., et al., *The hope and the hype of organoid research.* Development, 2017. **144**(6): p. 938-941.
8. Coll, M., et al., *Generation of hepatic stellate cells from human pluripotent stem cells enables in vitro modeling of liver fibrosis.* Cell stem cell, 2018. **23**(1): p. 101-113. e7.
9. Guan, Y., et al., *A human multi-lineage hepatic organoid model for liver fibrosis.* Nature communications, 2021. **12**(1): p. 6138.
10. Lee, C.A., et al., *Cryopreserved neonatal hepatocytes may be a source for transplantation: Evaluation of functionality toward clinical use.* Liver Transplantation, 2018. **24**(3): p. 394-406.
11. Afshari, A., et al., *Different approaches for transformation of mesenchymal stem cells into hepatocyte-like cells.* Stem cell research & therapy, 2020. **11**(1): p. 1-14.
12. Nitta, S., et al., *Conversion of mesenchymal stem cells into a canine hepatocyte-like cells by Foxa1 and Hnf4a.* Regenerative Therapy, 2020. **14**: p. 165-176.
13. Riveiro, A.R. and J.M. Brickman, *From pluripotency to totipotency: an experimentalist's guide to cellular potency.* Development, 2020. **147**(16): p. dev189845.
14. Dakhore, S., B. Nayer, and K. Hasegawa, *Human pluripotent stem cell culture: current status, challenges, and advancement.* Stem cells international, 2018. **2018**.
15. Sani, F., et al., *Differentiation of menstrual blood derived stem cell (MensSCs) to hepatocyte-liked cell on three dimensional nanofiberscaffold: poly caprolacton (PCL).* Journal of Biomedical Science and Engineering, 2016. **9**(04): p. 216.
16. Xing, X., H. Feng, and Z. Yuan, *Differentiation of bone mesenchymal stem cells into hepatocyte-like cells induced by liver tissue homogenate.* Genet. Mol. Res, 2016. **15**(3): p. 10.4238.
17. Cipriano, M., et al., *The role of epigenetic modifiers in extended cultures of functional hepatocyte-like cells derived from human neonatal mesenchymal stem cells.* Archives of toxicology, 2017. **91**: p. 2469-2489.

18. Eipel, C., K. Abshagen, and B. Vollmar, *Regulation of hepatic blood flow: the hepatic arterial buffer response revisited.* Annals of Gastroenterology & Hepatology, 2011. **9**(96).
19. Sambathkumar, R., et al., *Generation of hepatocyte-and endocrine pancreatic-like cells from human induced endodermal progenitor cells.* Plos one, 2018. **13**(5): p. e0197046.
20. Chen, J., et al., *The diversity and plasticity of adult hepatic progenitor cells and their niche.* Liver International, 2017. **37**(9): p. 1260-1271.
21. Liu, W., et al., *Clonal expansion of hepatic progenitor cells and differentiation into hepatocyte-like cells.* Development, Growth & Differentiation, 2019. **61**(3): p. 203-211.
22. Goulart, E., et al., *Adult and iPS-derived non-parenchymal cells regulate liver organoid development through differential modulation of Wnt and TGF-β.* Stem Cell Research & Therapy, 2019. **10**(1): p. 1-11.
23. Olgasi, C., A. Cucci, and A. Follenzi, *iPSC-derived liver organoids: a journey from drug screening, to disease modeling, arriving to regenerative medicine.* International journal of molecular sciences, 2020. **21**(17): p. 6215.
24. Kamishibahara, Y., et al., *Stabilized generation of human iPSC-derived liver organoids using a modified coating approach.* Biology Methods and Protocols, 2023. **8**(1): p. bpac034.
25. Rogers, A.D., *The biology of seamounts: 25 years on.* Advances in marine biology, 2018. **79**: p. 137-224.
26. Tachmatzidi, E.C., O. Galanopoulou, and I. Talianidis, *Transcription control of liver development.* Cells, 2021. **10**(8): p. 2026.
27. Yamashita, T., et al., *Billion-scale production of hepatocyte-like cells from human induced pluripotent stem cells.* Biochemical and biophysical research communications, 2018. **496**(4): p. 1269-1275.
28. Guicciardi, M.E., et al., *The spectrum of reactive cholangiocytes in primary sclerosing cholangitis.* Hepatology, 2020. **71**(2): p. 741-748.
29. Ogawa, M., et al., *Generation of functional ciliated cholangiocytes from human pluripotent stem cells.* Nature communications, 2021. **12**(1): p. 6504.
30. Zhang, Y., et al., *Crosstalk between NK cells and hepatic stellate cells in liver fibrosis.* Molecular Medicine Reports, 2022. **25**(6): p. 1-7.
31. Seo, W. and W.-I. Jeong, *Hepatic non-parenchymal cells: Master regulators of alcoholic liver disease?* World journal of gastroenterology, 2016. **22**(4): p. 1348.
32. MacParland, S.A., et al., *Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations.* Nature communications, 2018. **9**(1): p. 4383.
33. Schutgens, F. and H. Clevers, *Human organoids: tools for understanding biology and treating diseases.* Annual Review of Pathology: Mechanisms of Disease, 2020. **15**: p. 211-234.
34. Bar, S. and N. Benvenisty, *Epigenetic aberrations in human pluripotent stem cells.* The EMBO journal, 2019. **38**(12): p. e101033.
35. Hicks, M.R. and A.D. Pyle, *The emergence of the stem cell niche.* Trends in Cell Biology, 2022.
36. Vining, K.H. and D.J. Mooney, *Mechanical forces direct stem cell behaviour in development and regeneration.* Nature reviews Molecular cell biology, 2017. **18**(12): p. 728-742.

37. Akbari, S., et al., *Next-generation liver medicine using organoid models.* Frontiers in Cell and Developmental Biology, 2019. **7**: p. 345.
38. Huch, M. and H. Gehart, *van Boxtel R, Hamer K, Blokzijl F, Verstegen MM, et al.* Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell, 2015. **160**: p. 299-312.
39. Guan, Y., et al., *Human hepatic organoids for the analysis of human genetic diseases.* JCI insight, 2017. **2**(17).
40. Andersson, E.R., et al., *Mouse model of Alagille syndrome and mechanisms of Jagged1 missense mutations.* Gastroenterology, 2018. **154**(4): p. 1080-1095.
41. Nie, Y.-Z., et al., *Recapitulation of hepatitis B virus–host interactions in liver organoids from human induced pluripotent stem cells.* EBioMedicine, 2018. **35**: p. 114-123.
42. Baktash, Y., et al., *Single particle imaging of polarized hepatoma organoids upon hepatitis C virus infection reveals an ordered and sequential entry process.* Cell host & microbe, 2018. **23**(3): p. 382-394. e5.
43. Artegiani, B., et al., *Probing the tumor suppressor function of BAP1 in CRISPR-engineered human liver organoids.* Cell Stem Cell, 2019. **24**(6): p. 927-943. e6.
44. Yang, S., et al., *Liver three-dimensional cellular models for high-throughput chemical testing.* Cell Reports Methods, 2023. **3**(3).
45. Caiazza, C., S. Parisi, and M. Caiazzo, *Liver organoids: Updates on disease modeling and biomedical applications.* Biology, 2021. **10**(9): p. 835.
46. Nuciforo, S. and M.H. Heim, *Organoids to model liver disease.* JHEP Reports, 2021. **3**(1): p. 100198.
47. Wang, Y. and X. Wang, *Human ESC-derived Expandable Hepatic Organoids Enable Therapeutic Liver Repopulation and Pathophysiological Modeling of Alcoholic Liver Injury.* HPB, 2021. **23**: p. S46.
48. Younossi, Z., et al., *Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis.* Hepatology, 2019. **69**(6): p. 2672-2682.
49. Soret, P.-A., et al., *In vitro and in vivo models of non-alcoholic fatty liver disease: a critical appraisal.* Journal of clinical medicine, 2020. **10**(1): p. 36.
50. McCarron, S., et al., *Functional characterization of organoids derived from irreversibly damaged liver of patients with NASH.* Hepatology, 2021. **74**(4): p. 1825-1844.
51. Yuan, Y., et al., *Engineered Platforms for Maturing Pluripotent Stem Cell-Derived Liver Cells for Disease Modeling.* Cellular and Molecular Gastroenterology and Hepatology, 2023.
52. Heinecke, F., et al., *The offspring from rats fed a fatty diet display impairments in the activation of liver peroxisome proliferator activated receptor alpha and features of fatty liver disease.* Molecular and cellular endocrinology, 2020. **511**: p. 110818.
53. Wang, Y., et al., *Modeling human nonalcoholic fatty liver disease (NAFLD) with an organoids-on-a-chip system.* ACS Biomaterials Science & Engineering, 2020. **6**(10): p. 5734-5743.
54. Parola, M. and M. Pinzani, *Liver fibrosis: Pathophysiology, pathogenetic targets and clinical issues.* Molecular aspects of medicine, 2019. **65**: p. 37-55.
55. Bao, Y.-l., et al., *Animal and organoid models of liver fibrosis.* Frontiers in physiology, 2021. **12**: p. 666138.

56. Leite, S.B., et al., *Novel human hepatic organoid model enables testing of drug-induced liver fibrosis in vitro.* Biomaterials, 2016. **78**: p. 1-10.
57. Jiang, S., et al., *Development of a high-throughput micropatterned agarose scaffold for consistent and reproducible hPSC-derived liver organoids.* Biofabrication, 2022. **15**(1): p. 015006.
58. Kammala, A.K., et al., *Expression of CYP450 enzymes in human fetal membranes and its implications in xenobiotic metabolism during pregnancy.* Life Sciences, 2022. **307**: p. 120867.
59. Marquardt, J.U. and J.B. Andersen, *Liver cancer oncogenomics: opportunities and dilemmas for clinical applications.* Hepatic oncology, 2015. **2**(1): p. 79-93.
60. He, S., et al., *PDXliver: a database of liver cancer patient derived xenograft mouse models.* BMC cancer, 2018. **18**: p. 1-9.
61. Zhang, R., et al., *The biological process of lysine-tRNA charging is therapeutically targetable in liver cancer.* Liver International, 2021. **41**(1): p. 206-219.
62. Kim, J.H., et al., *Evolution of the multi-tRNA synthetase complex and its role in cancer.* Journal of Biological Chemistry, 2019. **294**(14): p. 5340-5351.
63. Baragaña, B., et al., *Lysyl-tRNA synthetase as a drug target in malaria and cryptosporidiosis.* Proceedings of the National Academy of Sciences, 2019. **116**(14): p. 7015-7020.
64. Broutier, L., et al., *Human primary liver cancer–derived organoid cultures for disease modeling and drug screening.* Nature medicine, 2017. **23**(12): p. 1424-1435.
65. Mun, S.J., et al., *Generation of expandable human pluripotent stem cell-derived hepatocyte-like liver organoids.* Journal of hepatology, 2019. **71**(5): p. 970-985.
66. Shinozawa, T., et al., *High-fidelity drug-induced liver injury screen using human pluripotent stem cell–derived organoids.* Gastroenterology, 2021. **160**(3): p. 831-846. e10.
67. Cheng, W., et al., *Polystyrene microplastics induce hepatotoxicity and disrupt lipid metabolism in the liver organoids.* Science of the Total Environment, 2022. **806**: p. 150328.
68. Van de Wetering, M., et al., *Prospective derivation of a living organoid biobank of colorectal cancer patients.* Cell, 2015. **161**(4): p. 933-945.
69. Hu, W. and M.A. Lazar, *Modelling metabolic diseases and drug response using stem cells and organoids.* Nature Reviews Endocrinology, 2022. **18**(12): p. 744-759.
70. van den Berg, C.W., et al., *Renal subcapsular transplantation of PSC-derived kidney organoids induces neo-vasculogenesis and significant glomerular and tubular maturation in vivo.* Stem cell reports, 2018. **10**(3): p. 751-765.
71. Huch, M., et al., *Long-term culture of genome-stable bipotent stem cells from adult human liver.* Cell, 2015. **160**(1-2): p. 299-312.
72. AlFatah Mansour, A., et al., *An in vivo model of functional and vascularized human brain organoids HHS Public Access Author manuscript.* Nat Biotechnol, 2018. **36**(5): p. 432-441.
73. Wang, Y.C., et al., *Cell reprogramming in liver with potential clinical correlations.* Journal of Digestive Diseases, 2022. **23**(1): p. 13-21.
74. Kondo, J. and M. Inoue, *Application of cancer organoid model for drug screening and personalized therapy.* Cells, 2019. **8**(5): p. 470.

75. Saito, Y., *Establishment of an organoid bank of biliary tract and pancreatic cancers and its application for personalized therapy and future treatment.* Journal of gastroenterology and hepatology, 2019. **34**(11): p. 1906-1910.
76. De Siervi, S., et al., *Patient-derived liver organoids as an in vitro model to study new personalized therapies targeting VDAC1 in intrahepatic cholangiocarcinoma.* Digestive and Liver Disease, 2023. **55**: p. S14.
77. Hendriks, D., et al., *Establishment of human fetal hepatocyte organoids and CRISPR–Cas9-based gene knockin and knockout in organoid cultures from human liver.* Nature Protocols, 2021. **16**(1): p. 182-217.
78. Zhan, T., et al. *CRISPR/Cas9 for cancer research and therapy*. in *Seminars in cancer biology*. 2019. Elsevier.
79. Ringel, T., et al., *Genome-scale CRISPR screening in human intestinal organoids identifies drivers of TGF-β resistance.* Cell stem cell, 2020. **26**(3): p. 431-440. e8.
80. Jeon, W., et al., *Evaluation of Radiation Sensitivity Differences in Mouse Liver Tumor Organoids Using CRISPR/Cas9-Mediated Gene Mutation.* Technology in Cancer Research & Treatment, 2023. **22**: p. 15330338231165125.
81. Zhang, Y.S., et al., *3D bioprinting for tissue and organ fabrication.* Annals of biomedical engineering, 2017. **45**: p. 148-163.
82. Bhise, N.S., et al., *A liver-on-a-chip platform with bioprinted hepatic spheroids.* Biofabrication, 2016. **8**(1): p. 014101.
83. Jin, Y., et al., *Vascularized liver organoids generated using induced hepatic tissue and dynamic liver-specific microenvironment as a drug testing platform.* Advanced Functional Materials, 2018. **28**(37): p. 1801954.
84. Schepers, A., et al., *Engineering a perfusable 3D human liver platform from iPS cells.* Lab on a Chip, 2016. **16**(14): p. 2644-2653.
85. Chung, M., et al., *Biomimetic model of tumor microenvironment on microfluidic platform.* Advanced healthcare materials, 2017. **6**(15): p. 1700196.
86. Wang, Y., et al., *In situ differentiation and generation of functional liver organoids from human iPSCs in a 3D perfusable chip system.* Lab on a Chip, 2018. **18**(23): p. 3606-3616.
87. Ingber, D.E., *Human organs-on-chips for disease modelling, drug development and personalized medicine.* Nature Reviews Genetics, 2022. **23**(8): p. 467-491.
88. Abady, M.M., et al., *The reprotoxic adverse side effects of neurogenic and neuroprotective drugs: current use of human organoid modeling as a potential alternative to preclinical models.* Frontiers in Pharmacology, 2024. **15**: p. 1412188.
89. Du, Y., et al., *A bile duct-on-a-chip with organ-level functions.* Hepatology, 2020. **71**(4): p. 1350-1363.
90. Chen, X., et al., *Organ-on-a-chip platforms for accelerating the evaluation of nanomedicine.* Bioactive Materials, 2021. **6**(4): p. 1012-1027.
91. Abady, M.M., J.-S. Jeong, and H.-J. Kwon, *Development and validation of an analytical method using liquid chromatography-tandem mass spectrometry for the therapeutic drug monitoring of seven cardiovascular drugs in clinical usage.* Journal of Chromatography B, 2023. **1214**: p. 123552.
92. Abady, M.M., J.-S. Jeong, and H.-J. Kwon, *Simultaneous quantification of 11 antiepileptic drugs using limited isotope-labeled internal standards in LC-*

MS/MS: An accuracy assessment. Journal of Chromatography B, 2024. **1240**: p. 124143.
93. Abady, M.M., J.-S. Jeong, and H.-J. Kwon, *Dried blood spot sampling coupled with liquid chromatography-tandem mass for simultaneous quantitative analysis of multiple cardiovascular drugs.* Journal of Chromatography B, 2024. **1242**: p. 124215.
94. Kogler, S., et al., *Organoids, organ-on-a-chip, separation science and mass spectrometry: An update.* TrAC Trends in Analytical Chemistry, 2023. **161**: p. 116996.
95. Lee, J., et al., *Advances in liver organoids: model systems for liver disease.* Archives of Pharmacal Research, 2022. **45**(6): p. 390-400.
96. Lieshout, R., et al., *Kinome profiling of cholangiocarcinoma organoids reveals potential druggable targets that hold promise for treatment stratification.* Molecular Medicine, 2022. **28**(1): p. 1-15.
97. He, C., et al., *Liver Organoids, Novel and Promising Modalities for Exploring and Repairing Liver Injury.* Stem Cell Reviews and Reports, 2023. **19**(2): p. 345-357.
98. Sun, X.-C., et al., *Liver organoids: established tools for disease modeling and drug development.* Hepatology Communications, 2023. **7**(4): p. e0105.
99. Liu, Y., et al., *A decade of liver organoids: Advancements in disease modeling.* Clinical and Molecular Hepatology, 2023.
100. De Siervi, S. and C. Turato, *Liver Organoids as an In Vitro Model to Study Primary Liver Cancer.* International Journal of Molecular Sciences, 2023. **24**(5): p. 4529.
101. Roman, G., et al., *iPSC-derived liver organoids and inherited bleeding disorders: Potential and future perspectives.* Frontiers in Physiology, 2023. **14**: p. 4.
102. Yang, X., et al., *Single-cell profiling reveals distinct immune phenotypes that contribute to ischaemia-reperfusion injury after steatotic liver transplantation.* Cell Proliferation, 2021. **54**(10): p. e13116.
103. Rossi, G., A. Manfrin, and M.P. Lutolf, *Progress and potential in organoid research.* Nature Reviews Genetics, 2018. **19**(11): p. 671-687.
104. Schneeberger, K., et al., *Large-scale production of LGR5-positive bipotential human liver stem cells.* Hepatology, 2020. **72**(1): p. 257-270.
105. Unagolla, J.M. and A.C. Jayasuriya, *Recent advances in organoid engineering: A comprehensive review.* Applied Materials Today, 2022. **29**: p. 101582.
106. Caralt, M., et al., *Liver bioengineering: from the stage of liver decellularized matrix to the multiple cellular actors and bioreactor special effects.* Organogenesis, 2014. **10**(2): p. 250-259.
107. Bernal, P.N., et al., *Volumetric bioprinting of organoids and optically tuned hydrogels to build liver-like metabolic biofactories.* Advanced Materials, 2022. **34**(15): p. 2110054.
108. Pang, Y., et al., *Organization of liver organoids using Raschig ring-like micro-scaffolds and triple co-culture: Toward modular assembly-based scalable liver tissue engineering.* Medical Engineering & Physics, 2020. **76**: p. 69-78.
109. Yin, X., et al., *Engineering stem cell organoids.* Cell stem cell, 2016. **18**(1): p. 25-38.
110. Park, S.E., A. Georgescu, and D. Huh, *Organoids-on-a-chip.* Science, 2019. **364**(6444): p. 960-965.

111. Li, J., et al., *The mechanisms and strategies to protect from hepatic ischemia-reperfusion injury.* Eur Rev Med Pharmacol Sci, 2015. **19**(11): p. 2036-2047.
112. Wang, K., et al., *Severity of early allograft dysfunction following donation after circulatory death liver transplantation: a multicentre study.* Hepatobiliary Surgery and Nutrition, 2021. **10**(1): p. 9.
113. DuBray Jr, B.J., et al., *Novel in vitro model for studying hepatic ischemia-reperfusion injury using liver cubes.* Surgery, 2012. **152**(2): p. 247-253.
114. Módis, K., et al., *Adenosine and inosine exert cytoprotective effects in an in vitro model of liver ischemia-reperfusion injury.* International Journal of Molecular Medicine, 2013. **31**(2): p. 437-446.
115. DeForest, C.A., B.D. Polizzotti, and K.S. Anseth, *Sequential click reactions for synthesizing and patterning three-dimensional cell microenvironments.* Nature materials, 2009. **8**(8): p. 659-664.
116. Ye, S., et al., *A chemically defined hydrogel for human liver organoid culture.* Advanced functional materials, 2020. **30**(48): p. 2000893.
117. Abady, M.M., et al., *Chapter 4 - Nanobiotechnology for the food industry: Current scenario, risk assessment, and management*, in *Nanobiotechnology for Food Processing and Packaging*, J. Singh, et al., Editors. 2024, Academic Press. p. 65-94.
118. Elella, M.H.A., et al., *Nanographites as Multidimensional Carriers for Advanced Therapeutic Applications*, in *Carbon Nanostructures in Biomedical Applications*. 2023, Springer. p. 67-93.
119. Saleh, S.R., et al., *Berberine nanoencapsulation attenuates hallmarks of scoplomine induced Alzheimer's-like disease in rats.* Current Reviews in Clinical and Experimental Pharmacology Formerly Current Clinical Pharmacology, 2021. **16**(2): p. 139-154.
120. Sato, T., et al., *Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium.* Gastroenterology, 2011. **141**(5): p. 1762-1772.
121. Kim, J., B.-K. Koo, and J.A. Knoblich, *Human organoids: model systems for human biology and medicine.* Nature Reviews Molecular Cell Biology, 2020. **21**(10): p. 571-584.
122. De Masi, C., et al., *Application of CRISPR/Cas9 to human-induced pluripotent stem cells: from gene editing to drug discovery.* Human genomics, 2020. **14**(1): p. 1-12.
123. De Crignis, E., et al., *Application of human liver organoids as a patient-derived primary model for HBV infection and related hepatocellular carcinoma.* Elife, 2021. **10**: p. e60747.