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Liver Organoids, Novel and Promising Modalities for Exploring and Repairing Liver Injury

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Abstract

The past decades have witnessed great advances in organoid technology. Liver is the biggest solid organ, performing multifaceted physiological functions. Nowadays, liver organoids have been applied in many fields including pharmaceutical research, precision medicine and disease models. Compared to traditional 2-dimensional cell line cultures and animal models, liver organoids showed the unique advantages. More importantly, liver organoids can well model the features of the liver and tend to be novel and promising modalities for exploring liver injury, thus finding potential treatment targets and repairing liver injury. In this review, we reviewed the history of the development of liver organoids and summarized the application of liver organoids and recent studies using organoids to explore and further repair the liver injury. These novel modalities could provide new insights about the process of liver injury.

Keywords Organoids · Liver · Injury · Repair · Mechanism · Model

Introduction

The incidence of liver disease is rising, accounting for approximately 2 million deaths per year worldwide [1]. Liver is the biggest solid organ, which plays a significant role in a variety of multifaceted physiological functions including metabolism, detoxification, and protein production [2–5], and it is susceptible to multiple kinds of injury and damage. The common factors induced liver injury and dysfunction include virus infection, alcohol, drug, ischemia–reperfusion, cancer, autoimmune diseases and metabolic disorder [6–12]. 2-dimensional (2D) cell line cultures and animal models are widely used for investigating the process of liver injury. However, due to the complex pathogenic mechanism of various liver injury, the traditional model cannot well simulate the injury process. Although 2D cell line cultures are established rapidly and conveniently, the lack of the organ structure and microenvironment cannot be its neglectable limitation, and the established 2D cell lines were highly genetically heterogeneous [13]. Animal models of acute liver failure tend to result in high mortality and are consuming in time, money and resources [14]. More importantly, the results of animal models may not be applicable to the situation in human body. Therefore, a new model is urgently needed to make up for these defects.

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During the past decades, great advances have been made in the technology of organoids [15, 16]. The term "organoid" is defined as a 3-dimentional (3D) structure, which is grown from stem or progenitor cells which self-organizes to recapitulate tissue architecture and specific functions in vitro [17, 18]. Organoids have the spatial structure, microenvironment and stable genetic background of primary tissue owing unique advantages (Table 1). The first organoid was derived from intestinal tissue using Lgr5 + stem cells in 2009 [19]. Later, liver organoids were established in 2013 [20, 21]. Liver organoids systems were gradually applied in many fields of biomedicine. As a self-renewing culture system, organoid has a broad prospect in regeneration medicine. In this review, we reviewed and summarized recent studies using organoids to explore and repair liver injury.

History of Liver Organoids

The term "organoid" has been proposed for decades. It used to be defined as a sub-cellular structure [22], and was used to describe tumors with complex structures as well [23]. Since the first intestinal organoid was established, organoid was gradually regarded as a 3D culture model in vitro. To bring clarity to the term "organoid", more than 60 experts representing 16 countries around the world reached a consensus that "organoid was a 3D structure derived from (pluripotent) stem cells, progenitor and/or differentiated cells that self-organize through cell–cell and cell–matrix interactions to recapitulate aspects of the native tissue architecture and function in vitro" [18].

The history of liver organoids can be traced back to 1985. Landry and colleagues used the spheroidal aggregate culture of hepatic cells isolated from newborn rat to cultivate a 3D culture of hepatocytes and bile duct-like cells [24]. Later, small hepatocytes (SHs), considered as progenitor cells [25, 26], were widely used in the studies of liver organoids [27, 28]. Generally, the currently widely accepted concept of organoids has been developing rapidly since 2013. Takebe and his colleagues first reported functional human liver organoids derived from induced pluripotent stem cells (iPSCs), human umbilical vein endothelial cells (HUVECs) and human mesenchymal stem cells (MSCs) [21], and modified this system using entirely iPSCs later [29]. Huch and colleagues established long-term 3D liver organoid of mouse and human adult biliary epithelial-derived progenitor cells [20, 30, 31]. Hu HL et al. cultured the hepatocytes-derived organoid system whose transcriptional profiles resembled those after partial hepatectomy [32]. In the same year, Peng WC et al. reported a liver organoids culture system derived from hepatocytes and found TNFα promoted and enabled long-term culture for more than 6 months [33]. Later, human embryonic stem cells (ESCs) and iPSCs were used to generates liver organoids which consisted of hepatocytes and cholangiocytes, recapitulating a functional bile canaliculi network [34]. These organoids were cultured in vitro to model liver disease or functional liver, and the functional liver organoids can be transplanted into mice, compensating for liver function. The comparison of current liver organoids was listed in Table 2.

In addition, the technology of organoids-on-a-chip was a milestone in the history of the liver organoids. This platform can control the biochemical microenvironment and nutrient supply using the technology of the microfluidically perfused biochip [37, 38]. And it was also used to investigate the multi-organ interactions [39]. It synergistically combined the best features of organoid and organ-on-a-chip, thus tending to develop a promising in vitro model [40, 41].

Recent years have witnessed considerable progress of liver organoids. This culture system was applied for pharmaceutical research [42–45], precision medicine, and modeling liver disease [34, 35, 45–51]. Additionally, liver organoids can also be used in exploring liver injury and repairing liver injury. This novel model could provide new insights about

Table 1 Comparison of in vitro model systems

Feature	Primary cell	Organoid	Cancer cell lines	PDX	Tumoroid
Success rate of establishment	+ + +	+ +	+ + +	+	+ +
Expansion	+	+ + +	+ + +	+ +	+ + +
Cost	+ +	+ +	+ + +	+	+ +
Drug screening	+	+ + +	+	+ +	+ + +
Genetic manipulation	+	+ + +	+ + +	+	+ + +
Recapitulation of primary tissue functions	+ + +	+ +	+	+ +	+ +
Disease model	+ +	+ + +	+	+	+
Regenerative medicine	+	+ + +	n.a	n.a	n.a
Spatial structure	+	+ + +	+	+ + +	+ + +

(+) possible, (+ +) suitable, (+ + +) best

PDX patient derived xenograft; *n.a.* not applicable

Table 2 Liver organoids models

Author	Year	Cell sources	Model	Functionality/Characteristics	Advantages	Limitation
Huch et al. [20]	2013	• mouse Lgr5 + liver stem cells	• functional liver	• LDL uptake • hepatocyte cytochrome p450 function • glycogen accumulation • albumin secretion • transplantation	• mature hepatocyte phenotype in vivo • contribution to liver function • long term expansion more than 12 months	• cannot reach a full rescue of the enzymatic defect • little engraftment after transplantation • lack of nonparenchymal cell • lack in vitro model
Takebe et al. [21]	2013	• human iPSCs • human MSCs • HUVECs	• functional liver	• albumin secretion • drug metabolism • vascularization • transplantation	• rescue liver failure in vivo • mimic liver ontogeny • multiple cell types	• different sources of cells • lack in vitro model
Huch et al. [30]	2015	• human adult bile-duct epithelial cells	• functional liver • A1AT deficiency • Alagille syndrome	• protein production • detoxifying function • bile acid production • transplantation	• long-term expansion • genetically stable • model inherited diseases • highly stable at the structural level	• lack of nonparenchymal cell
Hu HL et al. [32]	2018	• mouse hepatocytes • human hepatocytes	• functional liver	• albumin secretion • glycogen accumulation • lipid metabolism • transplantation	• recapitulate the proliferative damage-response • long-term expansion • genetically stable • significant graft expansion	• lack of nonparenchymal cell • lack in vitro disease model
Peng WC et al. [33]	2018	• primary hepatocytes	• functional liver	• albumin secretion • glycogen accumulation • LDL uptake • functional bile canaliculi • transplantation	• promote expansion in vitro • long term expansion more than 6 months • significant engraftment • mimic some aspects of in vivo liver regeneration • genetical manipulation	• lack of nonparenchymal cell • lack in vitro model
Ouchi et al. [35]	2019	• human PSC	• functional liver • steatohepatitis • fibrosis • Wolman disease	• cytochrome p450 3A4 function • Vitamin A storage ability • inflammatory response • lipid accumulation	• multiple cell types • measuring organoid stiffness reflects fibrosis severity	• the inter- and/or intra-batch variability
Mun SJ et al. [36]	2019	• human ESC • human iPSC	• functional liver • hepatic steatosis	• drug metabolism • detoxifying function • active mitochondrial bioenergetics • regenerative response	• long-term expansion • toxicity prediction • good viability after cryopreservation	• lack of nonparenchymal cell • low level of inflammatory response
Bin Ramli MN et al. [34]	2020	• human ESCs • human iPSCs	• cholestasis • NASH • functional liver	• albumin secretion • apolipoprotein B secretion • functional bile canaliculi • lipid metabolism • cytochrome P450 activity	• high-throughput • consistent in shape, size and structure • matrix-free	• lack of nonparenchymal cell

A1AT Alpha-1-Antitrypsin; *ESC* embryonic stem cells; *HUVEC* human umbilical vein endothelial cell; *iPSC* induced pluripotent stem cell; *LDL* low density lipoprotein; *MSC* mesenchymal stem cell; *NASH* non-alcoholic steatohepatitis

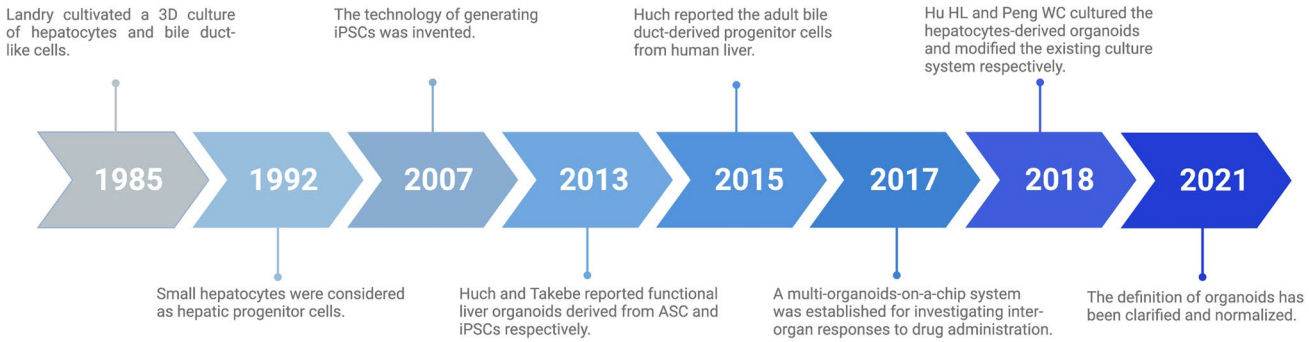

Fig. 1 History of liver organoids. The significant events in the history of the liver organoids are summarized chronologically. Abbreviations: 3D, 3-dimensional; iPSC, induced pluripotent stem cell; ASC, adult stem cell

the process of liver injury. The significant events in the history of the liver organoids are summarized chronologically in Fig. 1.

Liver Organoids and Stem Cells

Organoids can be cultivated from adult stem cells (ASCs) or pluripotent stem cells [52]. And pluripotent stem cells can be divided into ESCs and iPSCs [15]. Current studies on liver organoids are mostly based on the organoids grown from adult organ-specific progenitor cells and iPSCs, while the studies derived from human ESCs were limited [46].

The origin of liver stem cell was still controversial [53], and the prevailing school of thought was listed as follows. Huch et al. deemed that Lgr5 + cells induced by liver injury acted as liver stem cells, which were able to generate hepatocytes and bile ducts [20]. Sox9 or Epcam have long been regarded as biomarker of stem/progenitor cells [54, 55]. A population of periportal hepatocytes expressing low amounts of Sox9 was observed to regenerate the liver mass after injury [56]. Researchers also found that pericentral Axin2-positive stem cells with the ability to proliferate and self-renew to maintain hepatocytes homeostasis [57]. Lin et al. revealed that hepatocytes with high telomerase expression were distributed throughout the liver lobule, which were able to regenerate hepatocytes, self-renew and differentiate to hepatocyte clones [58]. Stem cells or progenitor cells is the origin of liver organoids. Lgr5 + cells were able to form organoids, while the ability to form organoids of other cell subsets and the difference between these derivative organoids awaits further investigation.

The technology of generating iPSCs has greatly promoted the study of organoids. In many aspects including morphology, ability of proliferating, gene expression, and telomerase activity, human iPSCs presented similarly to human ESCs [59]. Takebe and colleagues reported a functional human liver organ from iPSCs for the first time [21]. They cultivated three kinds of cells including hepatic endoderm cells from human iPSCs, HUVECs and human MSCs together to recapitulate the organogenetic interactions. Notably, after transplantation the liver organoids can generate vessels and connected quickly with host vasculature within 48 h [21]. This finding of organoids transplantation showed potential application to study regenerative medicine. Later, Takebe's team also generated successfully human liver buds entirely derived from iPSCs [29], dealing with the problem of figuring out and maintaining the suitable conditions for the different cells in the same coculture system.

To sum up, stem cells is the fundament for organoids research, thus the liver stem cell is highly needed to identified and clarified in the future. In addition, the process of culturing organoids revealed the proliferation and differentiation of stem cells, and liver organoids can recapitulate liver development in vivo to some degree [60].

Current Application of Liver Organoids

Pharmaceutical Research

Recently, pharmaceutical research is costing and inefficient, and clinical trials of many drugs that are effective in animals and cell lines have often failed, partially due to the traditional model cannot accurately recapitulate in vivo drug efficacy. Therefore, applying organoids in pharmaceutical research is a popular direction.

Organoids model have a promising potential in anti-cancer drug screening, which can be derived from cancer tissue and is called as tumoroids. Based on organoids model, a study reported that the ERK inhibitor SCH772984 was identified to be a potential therapeutic drug against primary liver cancer [61]. Nuciforo et al. used organoids derived from tumor needle biopsies to test sensitivity to sorafenib, which showed the potential treatment strategy for those patients who didn't undergo surgery [62]. In addition, another study

reported the model of hepatoblastoma organoids and found that JQ1 had more killing effects of tumoroids compared to adjacent non-tumor liver organoids [44]. However, there is a long way to go before putting the screening results of organoids into clinical practice, because some screening drugs were not approved for use in relevant disease. Although the result was convincing and reliable, caution should be exercised regarding off-label drug use, and side effects need to be considered.

Due to its complex pathogenesis, the genetic disease is hardly modeled. While, organoids inherit the genetic background of the primary tissue and recapitulate native tissue architecture and function to the greatest extent, which makes it become a direct and intuitive platform to explore disease mechanism and discovery drugs. A group established organoids model generated from patients with polycystic liver disease, and the experimental cystic fibrosis drug VX809 was found to increase cystic fibrosis transmembrane regulator (CFTR) function in vitro and may have therapeutic effect in cystic fibrosis [45].

To sum up, the pharmaceutical research of liver organoid mainly focuses on drug effectiveness and drug toxicity. Especially in drug discovery, a big-scale compound screening can be conducted based on liver organoids model. Liver organoids show their powerful ability to mimic in vivo condition, have more advantages compared to the other models and have a better application prospect.

Precision Medicine and Biobank

Due to the variance of individuals, the treatment effects of the same therapy strategy are inconsistent particularly in cancer cases. In recent years, precision medicine is highly being recommended to improve the prognosis and increase the therapeutic effect in clinical practices. However, there are two notable challenges in establishing precision models: maintaining the heterogeneity in different individual tumors and restructuring of the tumor microenvironment [63]. Organoids have been considered as the convincing and powerful preclinical model in precision therapy [64]. The organoids of a patient-specific tumor can be cultivated from primary liver cancer, and it can recapitulate the expression profile and the histology of the parental tumor, and retain the genetic alterations [61]. The common clinical drugs can be applied in organoids and the optimal treatment options can be decided after evaluating the efficacy and side effects. And specific therapy strategies can be determined according to the molecular subtypes of patients.

Building up a biobank containing abundant organoid samples is one of the future trends of applications. The biobank of rich samples can facilitate the establishment of screening platforms which cover a wide range of global populations genetic variance [65]. Biobanks of primary colorectal tumoroids had been established [66, 67]. The genetic features of the primary tumors and these histopathological features were stored and different molecular subtypes and clinical stages were covered. However, due to its unsatisfied success rate, the biobank of liver organoids was limited. A group cultivated liver tumor organoids from HCC needle biopsies, and it had about 26% success rate (10/38). There was not a statistically significant correlation between the clinically characteristics of patients and the success rate for generating organoids. On the contrary, there was a strong correlation with the histopathological condition and KI-67 [62]. Therefore, improving the success rate for generating organoids was also an urgent need in the process of building biobanks.

Exploring Liver Injury using Organoids

As mentioned above, the technology of liver organoids can be applied in pharmaceutical research and precision medicine, and each application has promising prospects. Additionally, the liver organoids can be used in exploring liver injury and finding potential treatment strategies. As reported previously, many organoids derived from organs except liver were already applied for exploring organ injury, including stomach [68], lung [69–71], intestine [72], heart [73], brain [74], kidney [75] and colon [71, 76] (Table 3). The liver organoids hold great promise for investigating liver injury, and the main reported studies are listed as follows.

Infection-induced Liver Injury

Hepatitis virus is the major cause of infection-induced liver injury. Hepatitis B virus (HBV) infection is not a neglectable challenge with over 240 million infected people globally [77], and the number of people infected with the hepatitis C virus (HCV) has reached 115 million [78]. Primary human hepatocytes (PHHs) were previously considered as the most suitable for hepatitis virus study [79]. However, it was difficult to obtain and maintain their growth. Establishing a novel and stable platform was needed. As far as we know, Nie and colleagues used iPSC-derived organoids to explore the HBV-induced liver injury for the first time. After infected by HBV, these liver organoids showed hepatic dysfunction, presenting down-regulation of hepatic gene expression, releasing early acute liver failure markers and alteration of hepatic ultrastructure [80]. Furthermore, liver organoids can be used for the study of hepatocarcinogenesis based on HBV-related liver injury [81, 82]. While, the researches using 3D cell culture systems to model HCV infection were more focused on HCV entry and revealed novel findings which were not seen in 2D cell lines [83, 84]. A recent study successful recapitulated the hepatitis E virus (HEV) infection in liver organoids and further found two potent HEV inhibitors [85].

Table 3 Exploring organ injury using organoids except liver

Authors	Organoids types	Injury types	Mechanisms and related pathways	Year	Reference
Bartfeld S et al	Stomach	Helicobacter pylori infection	Genes in the NF-κB signaling were upregulated during infection and the highest was human CGB	2015	[68]
Quantius J et al	Lung	Influenza virus infection	The movement of influenza virus towards distal lung stem cell niches is a pathogenic factor and impaired Fgfr2b signaling was considered as a potential mechanism	2016	[69]
Lu R et al	Intestine	Alcohol exposure	Alcohol exposure may result in intestine stem cell dysregulation, which played a role in alcohol-induced intestinal injury	2017	[72]
Voges HK et al	Heart	Cryoinjury	Collagenous fibrotic response and hypertrophy were not found in human cardiac organoid after cryoinjury treatment	2017	[73]
Daviaud N et al	Brain	Hypoxic injury	Apoptosis can be found in cerebral organoids after transient hypoxia. Outer radial glia and immature neurons suffered larger losses	2019	[74]
Digby JLM et al	Kidney	Cisplatin-induced injury	Cisplatin induced HAVCR1 and CXCL8 expression and caused DNA damage and cell death in the organoids. Organoid viability was impaired greatly by cisplatin	2020	[75]
d'Aldebert E et al	Colon	Inflammatory bowel diseases	IBD organoid cultures showed an inflammatory phenotype and tight junction proteins were also greatly decreased in IBD organoids	2020	[76]
Li X et al	Lung	Bleomycin-induced lung injury	After bleomycin treatment, lipid metabolism was downregulated and glucose metabolism was upregulated through autophagy in organoids for alveolar repair	2020	[70]
Han Y et al	Lung and Colon	SARS-CoV-2 infection	Lung organoids showed robust induction of chemokines and multiple colonic cell types express ACE2 and are permissive to SARS-CoV-2 infection. Drug screen found some drugs which were able to inhibit SARS-CoV-2 infection in lung organoids and colon organoids	2021	[71]

CGB chorionic gonadotropin beta; *IBD* inflammatory bowel diseases; *HAVCR1* hepatitis A virus cellular receptor 1; *CXCL8* C-X-C motif chemokine ligand 8; *SARS-CoV-2* severe acute respiratory syndrome coronavirus 2

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the pathogen responsible for the global pandemic of corona virus disease 2019 (COVID-19). The virus not only resulted in respiratory failure but also caused multi-organ injury including liver, gut, heart, and pancreas [86–88]. At present, the liver organoids have been applied in the study of SARS-CoV-2-induced liver injury [89, 90]. Zhao found these liver organoids were susceptible to SARS-CoV-2 and supported robust viral replication. The genes relating to tight junction formation and bile acid transportation were disordered and the relevant functions were impaired by SARS-CoV-2 infection [90]. These findings may better the understanding towards this disease and provide potential treatment strategies against COVID-19.

To sum up, these novel models provided a stable and reliable platform to explore infection-induced liver injury, and its 3D structure bettered the recapitulation of interaction between virus-cell and cell–cell during the process of infection.

Hypoxia-induced Liver Injury

Hypoxia is a pathological process of abnormal changes in tissue metabolism, function and morphology due to insufficient oxygen supply or oxygen use disorder. It is a common pathological state in various clinical diseases and even in medical treatment. Recently, a group established 15 organoids cultures derived from human liver biopsies [91]. The organoids were observed having functional ion channels including CFTR and anoctamin-1 (ANO1). This model was established to mimic hypoxia-related biliary injury during liver transplantation (LT). During hypoxia, the physiological function of CFTR and ANO1 was inhibited, and an AMP kinase inhibitor can restore the function of these two ion channels partially. Furthermore, the resistance of organoids to bile cytotoxicity was also reduced after hypoxia, thus increasing cell death. Accordingly, organoid is a powerful and reliable platform to study cholangiocyte anion exchange channels and their direct changes induced by hypoxia. In 3D cell culture systems, Liu et al. found that hypoxia was

capable of inducing EMT and cancer cell stemness through upregulating the expression of Twist1 and Bmi1 [92]. In conclusion, the new mechanism of hypoxia-induced liver injury can be discovered based on organoids model and the potential therapeutic strategies tend to be developed to repair injury.

Drug-induced Liver Injury

Drug-induced liver injury (DILI) is one of the critical challenges in drug development, and it is also associated with considerable morbidity and mortality [93]. Leite and colleagues cultivated liver organoids grown from HepaRG cells and Hepatic Stellate Cells (Hep/HSC) [94]. After a 24 h Acetaminophen (APAP) exposure, hepatocytes in the periphery of the organoid disappeared, and apoptotic cells remained surrounding the HSC core, showing the previously reported path of APAP-induced liver injury [95]. HSC-associated mRNAs including COL1A1, COL3A1 and LOXL2 were detected, presenting a dose-dependent up-regulation tendency only in Hep/HSC organoids and showing that HSC activation played a role in liver injury. The HSC activation role of APAP was confirmed in vivo in mice. They also detected the effects of pro-fibrotic compounds including Allyl alcohol and Methotrexate in liver organoids, and the organoids displayed fibrotic feature [94]. To sum up, these novel organoids were the first platform to study hepatocyte-dependent HSC activation, and provided a powerful tool to find underlying mechanism to prevent and reverse DILI.

Alcohol-induced Liver Injury

Excessive alcohol consumption is the leading cause of liver-related deaths in western countries. Oxidative stress and mitochondrial disorder were the major factors to promote steatosis in alcoholic liver disease (ALD) [7]. Based on the model of liver organoids, a study showed that after EtOH treatment for 7 days, the CYP2E1 activity was increased [49]. The up-regulated secretion of ALT, AST and LDH, and the reduction in cell viability revealed the damage in liver organoids. In addition, the gene expression of fibrogenic markers including LOXL2, COL1A1, COL3A1, ACTA2 and TGFβ1 were up-regulated, which showed the fibrogenic responses to EtOH in organoids model. The measurement of reactive oxygen species (ROS) and glutathione (GSH) in organoids revealed that oxidative stress was enhanced after EtOH treatment. In addition, inflammation also has been thought to play a key role in the pathogenesis of ALD [96]. The release of inflammatory mediators was measured in the EtOH-treated liver organoid, and both cytokines relative to chemokines and interleukins increased [49]. This reliable and practical in vitro system simulated the pathophysiology of ALD for the first time, and provided a powerful platform for exploring the underlying mechanisms as well.

Non-alcoholic Fatty Liver Disease

With the rise of patients with obesity and type 2 diabetes mellitus, non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease worldwide [97, 98], which progresses from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH), eventually severe liver fibrosis, cirrhosis and hepatocellular carcinoma [99, 100]. However, current NAFLD models based on PHHs and cancer cell lines cannot meet research needs, and the lack of appropriate in vitro human-based in vitro model hindered the deep understanding of NAFLD progression [101]. Mun SJ et al. reported a PSC-derived liver organoid model which recapitulated human hepatic steatosis phenotypes [36]. The model showed the functionality of lipid accumulation. However, it is insufficient to induce inflammation in the liver due to the lack of nonparenchymal cells. Ouchi and colleagues used human pluripotent stem cells to culture multi-cellular human liver organoids composed of hepatocyte-, stellate- and Kupffer-like cells which recapitulated key features of steatosis, inflammation and fibrosis phenotypes in a successive manner. Furthermore, they evaluated the fibrosis severity by measuring liver organoids stiffness [35]. Pingitore and colleagues cultured 3D spheroids composed by hepatocytes (HepG2) and hepatic stellate cells (LX-2) in an artificially determined ratio. When exposed to free fatty acids (FFA), the spheroids developed accumulations of fat and collagen, which can be rescued by liraglutide or elafibranor. Furthermore, based on organoids-on-chip system derived from iPSCs [41] or HepaRG [102], high-throughput drug screening potentially accelerated the NAFLD drug development. Based on NASH organoids model, Taesik et al. found that anti-CD47 antibody might be a new therapeutic option for NASH and liver fibrosis [103]. Ramli and colleagues used ESCs and iPSCs to establish liver organoids, which can be used to model NASH. After the incubation of FFA, the structure changes can be observed, including decay of bile canaliculi network and ductular reactions. The gene expression signatures of NASH organoid were similar to those of liver tissues from patients with NASH [34]. To sum up, these models recapitulated the spatial structure and its reaction toward FFA, while the cell types were limited. The ideal NASH model should contain hep-orgs, stellate- and Kupffer cells, which played a key role in the progression of NAFLD [104].

Application of Organoids in Regenerative Medicine

As mentioned above, the model of liver organoids was a reliable and stable platform for exploring liver injury, which shed more light on new treatment strategies. Apart from this

perspective, the liver organoids grown from in vitro systems can be used to repair liver injury in regenerative medicine.

The ideal pattern of regenerative medicine contains three steps: culturing tissues or organs in vitro, transplanting functional tissue into body and correcting the pre-existing disorder. The cell from a healthy donor or the gene edited cell from patients may be the sources of organoids for regenerative medicine purposes [65]. Transplantation of in-vitro-generated organoids is an application with great prospects, and its feasibility is the primary and basic consideration. And vascularization and immune response are critical to success rate of transplantation. Takebe et al. reported that after transplanting into nude mice, the organoids could reconstruct functional vessels and rescued the drug-induced liver injury [105]. A group successfully transplanted human iPSC-derived liver organoids into immunocompetent mice by encapsulating organoids in biocompatible hydrogel capsules [106]. These capsules were able to prevent direct immune cell rejection but it did not mean the total elimination of immune response. Although transplantation in animal models mimics the condition in vivo, it did not always predict the real outcomes in practice [107]. Notably, Sampaziotis and colleagues reported a groundbreaking finding in 2021 [108]. Gallbladder organoids were injected into the intrahepatic ducts of human livers and then delivered into a terminal branch of intrahepatic ducts. The bile PH was measured less than 7.5 before the experiment, which meant ischemic duct injury [109]. After treatment, the pH

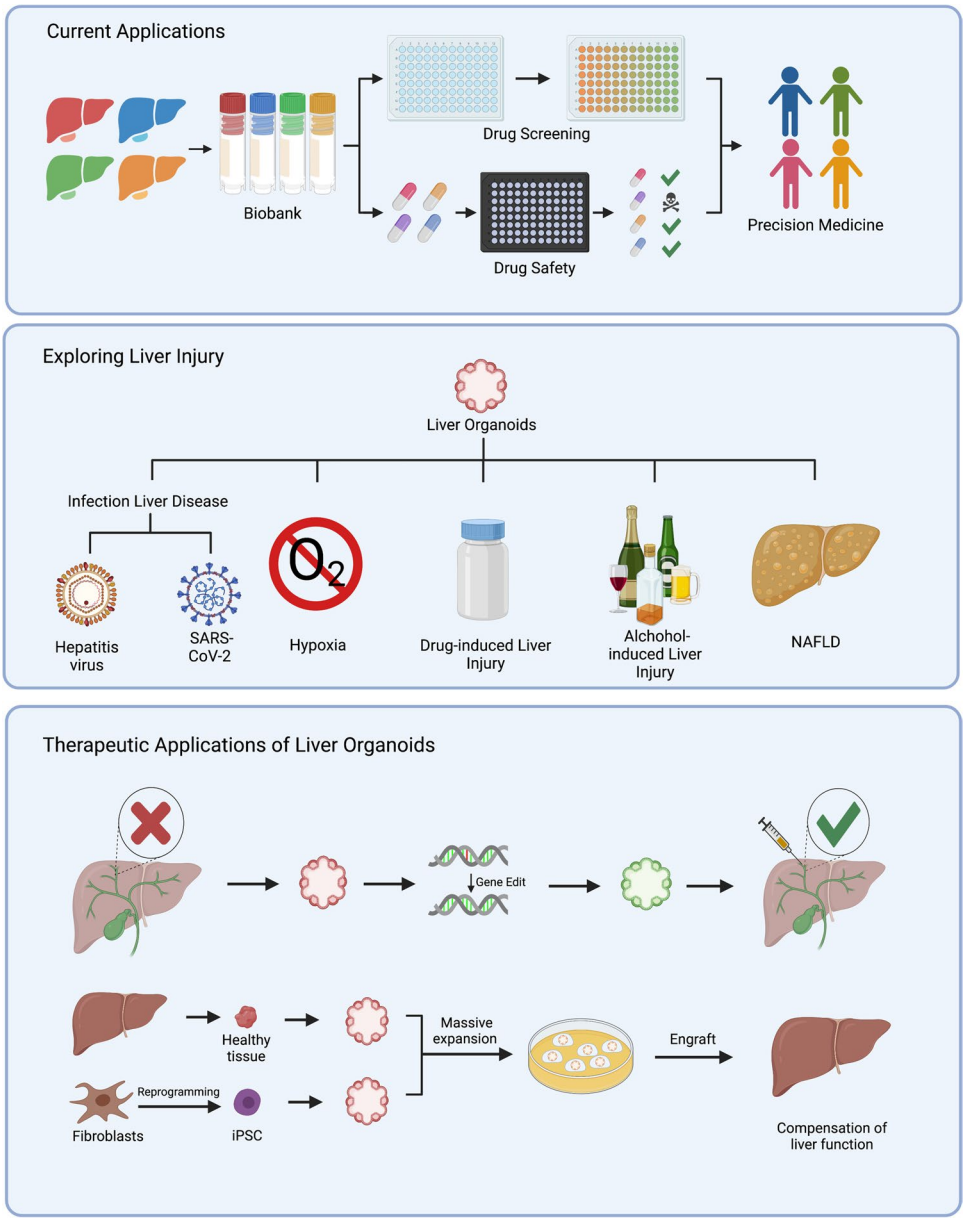

Fig. 2 Applications of liver organoids. The current application of liver organoids mainly focuses on establishing the biobank of organoids and testing drug effectiveness and drug toxicity. Some studies used organoids to constructed diseases models in vitro and exploring the underlying mechanism of relevant diseases. Furthermore, genetically modified organoids can be used as a source for regenerative medicine purposes. In addition, the liver organoids can expand massively in vitro and be transplanted back for compensating the liver function. Abbreviations: NAFLD, non-alcoholic fatty liver disease; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

and volumes of bile from injected duct were higher, and the injected duct did not show evidence of cholangiopathy, which meant that organoids could maintain their function and repair bile ducts injury [108].

To sum up, organoid technology showed a lot of prospects in regenerative medicine. The future study can apply liver organoids to release the biliary complication, improve liver function of the Small-for-size Syndrome and better the quality of expanded criteria donors for expanding the liver donor pool, though there is a long way to go before the mature clinical application.

Future Direction

Recent years have witnessed great advances in the field of liver organoids. Enormous studies have revealed the promising potential of organoids in biomedicine researches including disease modeling, mechanisms exploring, precision medicine, regenerative medicine and pharmaceutical researches (Fig. 2) [17, 63, 65, 108, 110]. However, this technology also has its drawbacks and limitations, and possible solutions are also discussed below.

Firstly, there are some stumbling blocks in the process of cultivating organoids. Establishing adult stem cell-derived liver organoids needs fresh patient-origin tissue from surgical resection or needle biopsy, and the availability of patient tissue may be limited. And the amount of freshly-isolated hepatocytes may be limited due to the tissue size, and the amplification of large number of cells in spinner flasks may be cost-prohibitive [111]. Remarkably, the success rate of establishment of liver organoids is a matter of urgent concern to us, however it is lower than those of other organ organoids to some extent. In addition, although the main steps to grow liver organoids are similar in different laborites, there are still not standardized and consensual protocols, especially in terms of what growth factors are used to induce differentiation, which may result in different cell maturity further leads to deviation of results. Matrigel is the most commonly utilized matrix, and the ingredients are complex and heterogeneous. Therefore, the appropriate extracellular matrix also needs to be identified [112]. Currently, a group reported a nanofibrillar hydrogel with controllable stiffness for organoids culture [113], and it is highly needed to exploit a standard biomimetic material of known composition in the future.

Secondly, the ability of mimicking the real organs can be further improved. Although liver organoids show great advantages in cell types and spatial structures compared with conventional 2D cultures, it cannot reproduce every cell type in liver tissue and simulate the real and dynamic cell–cell interactions totally. The nature of their random growth makes it difficult to precisely control their shape and microenvironment, which limits their use in the clinic and lab [114, 115]. Fortunately, a current study developed bioengineering strategies to induce the deterministic formation of intestinal organoids [116]. The standard and uniform liver organoids are awaiting further investigation. In addition, the immune-checkpoint inhibitors have revolutionized the clinical guidelines of HCC treatment in the past 5 years [117]. The organoid derived from HCC lacks the immune microenvironment, and this has greatly hindered the understanding on the mechanism of immune responses and evasion. Co-culture of organoids with immune-cells may be a promising solution to deal with the problem.

Thirdly, the variety of models based on liver organoids can be much richer, and organoids-on-a-chip can simulate more disease models in vitro. Although there are already some novel models based on organoids for exploring liver injury, the amount of these models is still limited compared with animal models and traditional 2D cell models. IRI is a significant type of liver injury and often occurs after surgical procedures such as hepatic resection and LT [118]. As far as we concerned, IRI is an important risk factor of early allograft dysfunction, resulting in the failure of LT [119, 120]. A deep understanding of IRI remains challenging, and the mechanism of IRI is still not well understood [121–123]. At present, researchers investigate the mechanism of IRI by animal models and the in vitro model of IRI [124, 125]. However, the emergence and progress of organoids-on-a-chip gives it more possibility to refine existed models and establish more disease models in vitro. The most remarkable advantages are its controllable microenvironment and nutrient supply using the technology of the microfluidically perfused biochip. Theoretically speaking, the technique is able to mimic the process of ischemia and reperfusion. However, an in vitro model of liver IRI has not been established in organoids. We hold the view that with the development of organoids-on-a-chip technology, the organoid model of liver IRI may be successfully established in the near future and widely applied.

In conclusion, as a newly developed platform, organoid has great application potential and prospects, although it is still not perfect and needs to be improved in some aspects. In the future, it is prone to be a usual and powerful model for biomedicine researches.

Abbreviations ALD: Alcoholic liver disease; ANO1: Anoctamin-1; APAP: Acetaminophen; ASCs: Adult stem cells; CFTR: Cystic fibrosis transmembrane regulator; COVID-19: Corona virus disease 2019; DILI: Drug-induced liver injury; ESCs: Embryonic stem cells; FFA: Free fatty acids; GSH: Glutathione; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HEV: Hepatitis E virus; HSCHep/HSC: HepaRG cells and hepatic stellate cells; iPSC: Induced pluripotent stem cell; IRI: Ischemia-reperfusion injury; LDL: Low density lipoprotein; LT: Liver transplantation; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; PHHs: Primary human

hepatocytes; ROS: Reactive oxygen species; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; SHs: Small hepatocytes; TNF: Tumor necrosis factor; 2D: 2-Dimensional; 3D: 3-dimensional

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Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Declarations

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