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RESEARCH ARTICLE

Modeling Liver Fibrosis Using hiPSC-Derived Liver Organoids: Methods and Applications

Faris Ksatrianto,[†] Deviana Lavender Suhanto,[†] Rizal Azis,[*‡] Reyhan Natadilandes,[¶] Raudina Qamarani,[¶] Aris Muhammad Nurjamil,[¶] and Wahyu Widowati[§]

†Biomedical Technology, Department of Electrical Engineering, Faculty of Engineering, Universitas Indonesia, Depok, Indonesia
‡Biomedical Engineering, Department of Electrical Engineering, Faculty of Engineering, Universitas Indonesia, Depok, Indonesia
¶Biomolecular and Biomedical Research Center Bandung, Aretha Medika Utama, West Java 40163, Indonesia
§Faculty of Medicine, Maranatha Christian University, West Java 40164, Indonesia
*Corresponding author. Email: rizal@eng.ui.ac.id

Abstract

Liver fibrosis represents a pathological state characterized by the excessive deposition of extracellular matrix, which is a consequence of persistent hepatic injury. While various therapeutic interventions, including antiviral treatments and modifications to lifestyle, are available, a definitive cure remains elusive. This review intends to examine in detail how human-induced pluripotent stem cells (hiPSCs) contribute to liver fibrosis modeling by elucidating their directed differentiation into hepatocytes, hepatic stellate cells, and endothelial cells, as well as their role in 2D co-culture systems and 3D liver organoids. The value of these models is considered through their skill in reproducing fibrotic disease mechanisms, facilitating trials of antifibrotic therapies, and fostering the establishment of customized therapeutic options. In addition to delineating the merits of hiPSC-derived organoids—such as scalability, patient specificity, and ethical viability—this review also addresses prevailing challenges, including inadequate vascularization, absence of immune components, and variability in maturation and reproducibility across different platforms. Resolving these issues is imperative for augmenting the translational value of hiPSC-derived liver organoids in fibrosis analysis and the innovation of treatment alternatives.

Keywords: Antifibrotic, Disease Modeling, Hepatic Fibrosis, Human iPSCs, Liver Organoids

1. Introduction

The liver, a vital organ situated in the right upper quadrant of the body beneath the diaphragm, is responsible for the production of essential proteins, detoxification, and numerous metabolic processes. It produces enzymes that facilitate digestion, eliminates noxious substances, and processes a variety of metabolites. Furthermore, the liver plays a central role in regulating bile acid and cholesterol metabolism, detoxifying endogenous and exogenous compounds, and storing glucose—functions that are essential for maintaining systemic homeostasis. Figure 1 illustrates the key physiological roles of the liver, including its involvement in macronutrient metabolism, detoxification, and biosynthesis of plasma components. The body's numerous systems are supported by these hepatic functions, including hormone regulation and lipid turnover [1]. The liver's capacity to execute these functions is compromised when it malfunctions, which can have an effect on the body's overall health [2].

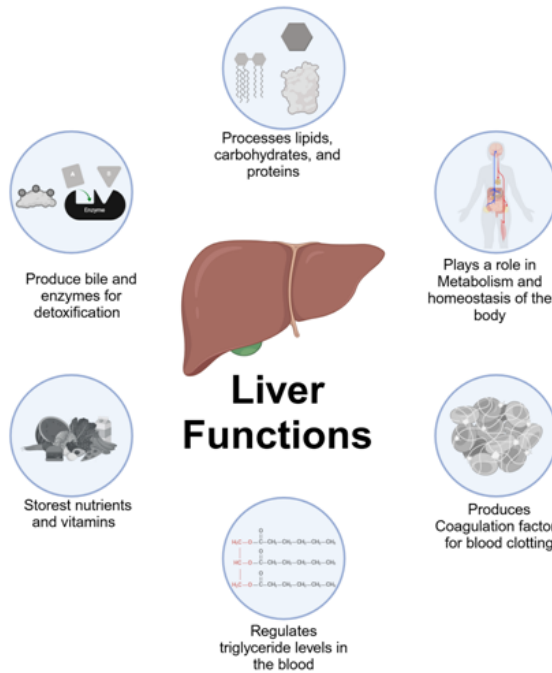

Figure 1. The illustration of key physiological functions of the liver, including macronutrient metabolism, detoxification, nutrient storage, bile production, and synthesis of coagulation factors.

These processes are essential for maintaining systemic homeostasis and supporting vital functions such as digestion, immune regulation, and metabolic balance.

Liver disease constitutes a significant global health challenge, responsible for more than two million fatalities each year—approximately four percent of worldwide mortality, equivalent to one in every 25 deaths [3]. Liver pathologies are systematically classified according to their etiological mechanisms into acute liver injury (ALI), viral hepatitis, alcoholic liver disease (ALD), metabolic-associated fatty liver disease

(MAFLD), liver fibrosis (LF), cirrhosis, and hepatocellular carcinoma (HCC). [4]. Among these classifications, liver fibrosis is marked by abnormal wound healing processes and the excessive accumulation of extracellular matrix (ECM), which progressively compromises liver architecture and functionality [5]. In situations lacking fitting therapeutic solutions, fibrosis has the potential to advance to cirrhosis, hepatic failure, or liver tumor. [6].

On a global scale, liver fibrosis impacts an estimated 3.3% of the population, while cirrhosis exhibits a prevalence of approximately 1.3% [5]. Cirrhosis ranks as the 11th leading cause of mortality worldwide, contributing to nearly two million deaths each year. The mortality associated with cirrhosis has escalated markedly, increasing from 899,000 in 1990 to over 1.32 million in 2017 [7], [8]. The transition from fibrosis to cirrhosis is frequently characterized by an insidious and asymptomatic trajectory, thereby complicating early diagnostic efforts. Consequently, the early identification of liver disease during initial or pre-cirrhotic phases is imperative for enhancing patient outcomes. Risk factors correlated with fibrosis encompass advanced age, male gender, tobacco use, obesity, hypertension, dyslipidemia, type 2 diabetes mellitus, fatty liver disease, abnormal liver function tests (LFTs), hepatitis B surface antigen (HBsAg) positivity, and metabolic syndrome [9].

The progression of liver fibrosis can be slowed by a variety of current interventions, including weight loss and exercise. Certain treatments are available for specific causes of liver fibrosis, including the use of ACE inhibitors (e.g. benazepril, lisinopril, and ramipril) in chronic liver disease [10] and antiviral medicines (e.g. Epclusa, Harvoni, and Mayvret) in the treatment of hepatitis C virus infection [11]. Despite the fact that there is currently no entirely effective treatment for liver fibrosis, research on the mechanism of liver fibrosis progression indicates that the potential to reverse this condition exists by eliminating pathogens or addressing the underlying cause of the disease, such as curing viral infections [6]. Furthermore, in contemporary years, a plethora of in vitro and in vivo experimental models have been established to advance the formulation of antifibrotic pharmaceuticals that exhibit both safety and efficacy. Currently, there is a lack of approved pharmacotherapy for treating liver fibrosis, notwithstanding the broadening spectrum of treatment strategies accessible for this health concern [5].

Liver transplantation represents a final therapeutic intervention for individuals with advanced liver fibrosis and cirrhosis, given that chronic liver disease may result in significant complications that elevate morbidity and mortality risks. In advanced stages, the liver's functional capacity is compromised, rendering transplantation the sole option for prolonging the patient's life expectancy [12]. Liver transplantation as a treatment option presents notable limitations. Organ availability represents a significant limitation, as the demand exceeds the supply of available organs. Liver transplantation involves risks of post-operative complications, including organ rejection, infection, and other health issues, which may impede recovery and negatively impact long-term patient outcomes. The high costs associated with surgery, follow-up care, and management of post-operative complications present significant barriers to accessing the procedure [13]. Current therapies for liver fibrosis frequently fail to produce satisfactory outcomes, particularly in severe instances. Consequently,

there is a necessity for innovative therapies and the advancement of more effective and safer anti-fibrotic agents to enhance treatment success and tackle the complexities associated with liver fibrosis [14].

In organoid engineering research, induced pluripotent stem cell (iPSC)-derived liver organoids have recently gained significant attention. Because they combine the capacity for self-organization with the possibility for specialized development towards particular cell types or tissues, organoids based on iPSCs are thought to offer benefits over organoids formed from adult tissues [15]. Exposure to selected mediators allows pluripotent stem cells, known as human induced pluripotent stem cells (hiPSCs), to shift from somatic cells into an undifferentiated condition. Like embryonic stem cells, hiPSCs are endowed with the intrinsic skill to continuously renew and morph into assorted types of somatic cells. [16]. HiPSCs are a valuable tool in regenerative medicine because of their capacity to self-renew and differentiate into diverse cell types [17]. Thus, it is anticipated that hiPSCs will contribute to the creation of more novel and targeted treatments to restore damaged tissues or organs, thus broadening the use of regenerative medicine in the future [18].

The advancement of iPSC technology has emerged as a promising source of stem cells for cell treatment in organ fibrosis. Cells produced from induced pluripotent stem cells (iPSCs), comprising epithelial cells, macrophages, and endothelial cells, demonstrate the potential to heal damaged tissues and stimulate regeneration in several organs impacted by fibrosis. [19]. The iPSCs can be differentiated into hepatocyte-like cells that exhibit functional and phenotypic characteristics of mature adult hepatocytes, making them a valuable in vitro model for studying liver physiology and pathology. This model enhances gene expression and enzyme activity related to liver function, encompassing enzymes implicated in drug metabolism and detoxification [20]. Moreover, iPSCs can develop into hepatic stellate cells (HSCs) that mimic primary human HSCs regarding phenotype and functionality, including their capacity to activate in response to damage. This approach enables iPSC-HSCs to elicit biologically pertinent fibrogenic and toxic responses [21]. Image 1 elucidates the hepatic dysfunctions associated with liver disease. The capacity of iPSCs to promote tissue regeneration and substitute damaged liver cells presents opportunities for more effective, realistic, and creative treatments for liver fibrosis. This paper aims to elucidate the role of iPSCs in liver fibrosis modeling and assess the potential of this technology to enhance the precision of disease modeling and facilitate the development of more effective and targeted therapies.

2. Liver as an Organ

The liver, recognized as the most substantial internal organ, fulfills a critical function in metabolic processes, detoxification, immunological responses, and the storage of nutrients [22]. The liver's primary structural entity is the hepatic lobule, which consists of hepatocytes arranged around a central vein and sinusoidal capillaries that enable both blood circulation and molecular interchange [23], [24]. The liver's cellular composition is primarily parenchymal cells—mainly hepatocytes, comprising 80%—alongside non-parenchymal cells (NPCs), which feature liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs), Kupffer cells (KCs), and cholan-

giocytes. [25], [26]. Hepatocytes execute vital functions, including the synthesis of bile, protein production, and the metabolism of pharmaceuticals, whereas NPCs play a role in regulating immune mechanisms, maintaining structural integrity, and facilitating intercellular communication [27], [28].

In fibrosis, hepatocyte damage triggers immune and inflammatory pathways involving DAMPs, reactive oxygen species (ROS), TNF-α, and TGF-β, thereby activating KCs and HSCs [29], [30]. HSC activation results in their conversion to myofibroblasts that synthesize ECM components, leading to collagen accumulation and fibrotic tissue remodeling. [31]. LSECs also experience capillarization, resulting in the loss of fenestrae and contributing to the advancement of fibrosis [32]. This phenomenon engenders a cycle of injury wherein inflammation and ECM deposition exacerbate hepatocellular injury [33]. The infiltration of immune cells—such as monocytes and lymphocytes—further fosters a pro-fibrotic milieu [34], while alterations in ECM characteristics modify tissue stiffness, thereby intensifying mechanotransduction-related pathways associated with fibrosis [35].

The liver's involvement in lipid and carbohydrate metabolism, protein synthesis, and detoxification is paramount to the maintenance of homeostasis. This encompasses functions such as glycogen storage, gluconeogenesis, the urea cycle, and the secretion of bile for lipid digestion [36], [37], [38]. Enzymatic systems such as CYP450 are instrumental in the biotransformation of xenobiotics and pharmaceuticals and any disruptions to these systems can lead to systemic toxicity [39], [40]. This streamlined summary gives essential insights for understanding the cellular and molecular operations that are vital to liver fibrosis, thereby reinforcing the importance of human induced pluripotent stem cell (hiPSC)-derived liver frameworks.

3. Liver Fibrosis Pathogenesis

The complex mechanism underlying hepatic fibrosis is characterized by an abnormal accumulation of extracellular matrix (ECM) constituents, predominantly collagen, subsequent to hepatic injury. [41]. Hepatocytes (PCs) and non-parenchymal cells (NPCs), especially HSCs and portal fibroblasts (PFs), interact dynamically. Hepatocytes secrete cytokines and growth factors that activate HSCs early in liver damage, promoting fibrogenesis. In response to liver injury, hepatocytes release pro-inflammatory cytokines such TGF-β and IL-6, which play crucial roles in activating HSC and PF [42]. Activated HSCs become myofibroblast-like cells that synthesize and deposit collagen and other ECM components [43]. Cytokine-mediated signaling pathways enhance cell proliferation and migration to the damage site [44].

The triggering of hepatic stellate cells (HSCs) represents a critical moment in the escalation of liver fibrosis. Activated HSCs are recognized as the principal origin of myofibroblasts across various liver injury scenarios, including toxin-mediated liver pathologies, biliary dysfunctions, and steatosis liver disease. [45]. Myofibroblasts play a crucial role in the fibrotic response by producing collagen and other extracellular matrix proteins, which results in the formation of fibrous tissue that can impair normal liver architecture and function [46]. Additionally, PFs contribute to the initial deposition of ECM during liver injury [47].

Other NPCs including liver sinusoidal endothelial cells (LSECs) and Kupffer cells

(liver macrophages) also perform fibrogenic functions. Additional mediators from these cells can boost HSC activation and fibrosis [23]. These cell types combine to form a milieu that favors fibrogenesis, with persistent inflammation and sustained tissue repair [48]. Excess ECM, especially collagen, causes fibrosis, which can lead to cirrhosis if the injury is not treated [49]. A thorough understanding of PCs and NPCs in liver damage and fibrosis is necessary to develop tailored therapies to minimize hepatic fibrosis and associated consequences.

The study of liver fibrosis stands to benefit significantly from the implementation of liver organoids derived from human induced pluripotent stem cells (hiPSCs). These organoids present a more physiologically pertinent system compared to traditional two-dimensional cell cultures, as they more accurately emulate the three-dimensional structure, multicellular composition, and dynamic microenvironment characteristic of native hepatic tissue. Notably, hiPSC-derived liver organoids demonstrate the capability for self-renewal and proliferation, facilitating their application in high-throughput toxicological assessments and extensive compound screening within both pharmaceutical and industrial domains [50]. Also, by reflecting vital properties of liver activities and configuration, these organoids yield strong in vitro representations for studying liver illnesses such as fibrosis and non-alcoholic fatty liver disease (NAFLD). Additionally, the challenges associated with the acquisition, viability, and ethical implications of primary human liver cells can be circumvented through hiPSC-based systems, which offer a renewable and scalable cellular resource for disease modeling and therapeutic advancements [51].

By exposing hiPSC-derived liver organoid models to specific substances such as thioacetamide (TAA) or free fatty acids (FFA), fibrogenesis can be experimentally induced. This exposure leads to measurable changes in inflammatory markers, fibrosis-related gene expression, and liver function, making it a valuable approach for studying the progression of liver disease [52]. Additionally, hiPSC-based therapies have shown promise in attenuating fibrosis through multiple mechanisms for example, embedding these organoids into experimental animal systems has been found to correlate with increased survival rates and the recovery of liver function, largely due to the engagement of M2 macrophages that are vital in driving antifibrotic mechanisms. This capacity for regeneration renders hiPSC-derived organoids a valuable element in the formulation of therapeutic interventions for liver fibrosis [53]. The complex interactions between various liver cell types, as well as the process of hepatic fibrogenesis, are illustrated in Figure 2.

Compromised hepatocytes release inflammatory mediators that activate Kupffer cells, which subsequently secrete cytokines such as TGF-β and TNF-α. These mediators precipitate the trans differentiation of quiescent HSCs into myofibroblast-like cells, which represent the principal source of extracellular matrix (ECM) proteins. This cascade engenders excessive ECM accumulation, disruption of hepatic architecture, and the progression of fibrosis.

4. The Use of hiPSC for Liver Fibrosis Modelling

Liver fibrosis denotes the excessive deposition of extracellular matrix constituents resulting from chronic hepatic injury, and it persists as a significant global health issue.

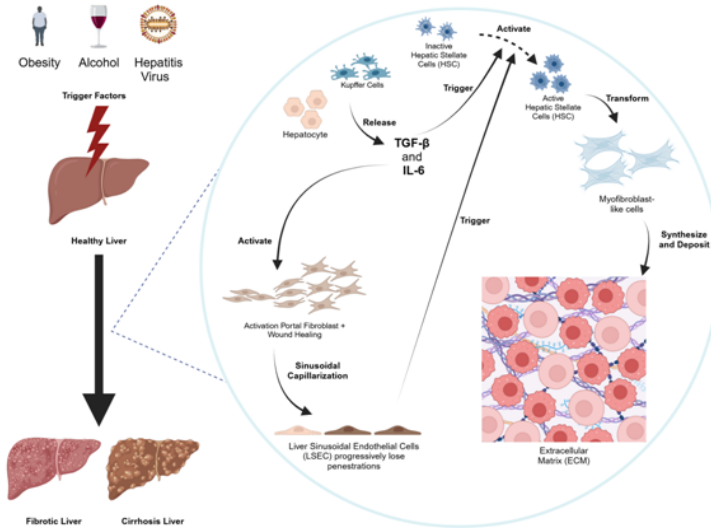

Figure 2. Representative illustration of hepatic fibrosis evolution emphasizing the interaction between hepatocytes, Kupffer cells, and hepatic stellate cells (HSCs) in response to persistent hepatic injury

Longstanding medical strategies have primarily been directed towards dealing with the origins of liver ailments, encompassing metabolic syndromes, alcohol dependency, and hepatitis of viral origin. Though modern interventions—like ongoing antiviral drugs for hepatitis B and direct-acting antiviral agents (DAAs) for hepatitis C—have demonstrated effectiveness in improving fibrosis and liver histology [54], they are frequently limited by long treatment periods and possible adverse impacts, which can jeopardize patient adherence and overall treatment efficacy [14]. Additionally, while liver biopsy is commonly accepted as the conclusive measure for gauging fibrosis, it is invasive, entails procedural hazards, and is not consistently achievable in typical clinical situations [55].

Human induced pluripotent stem cells, or hiPSCs, have transformed the modeling of liver disorders, particularly fibrosis, in recent years. HiPSCs can be produced from easily accessible tissues and can differentiate into hepatocyte-like cells that closely resemble the physiological properties of natural liver cells, in contrast to conventional cell sources such primary hepatocytes or cancer cell lines [56]. A greater comprehension of the disease mechanisms and the discovery of new treatment targets are made possible by this capability, which makes it possible to create patient-specific models that replicate the pathophysiology of liver fibrosis. A major advantage over primary cells, which have a limited lifespan, is that hiPSCs possess enhanced self-renewal capability, enabling long-term expansion in vitro. [57].

The application of hiPSCs in liver fibrosis modeling has facilitated the investigation of the cellular and molecular pathways associated with fibrogenesis. The activation of hepatic stellate cells (HSCs) is essential for fibrosis progression [58]. Investigations can be conducted on the interactions and responses of hiPSC-derived hepatocytes

and HSCs to stimuli such as inflammatory cytokines and extracellular matrix components.[59]. This method improves our comprehension of the fibrotic process and facilitates the evaluation of potential antifibrotic compounds in a controlled setting.

While traditional treatments for liver fibrosis have advanced, many still require invasive procedures and are limited by poor patient adherence, often due to prolonged treatment durations or adverse effects [60]. The application of hiPSCs in liver fibrosis studies offers potential for targeted and effective treatments. By using the unique benefits of hiPSCs, scientists can expand their knowledge of the complex processes behind liver fibrosis and open the door to the creation of novel treatment strategies. Figure 3 illustrates the use of hiPSC-derived liver cells in 2D and 3D culture systems to model fibrosis progression.

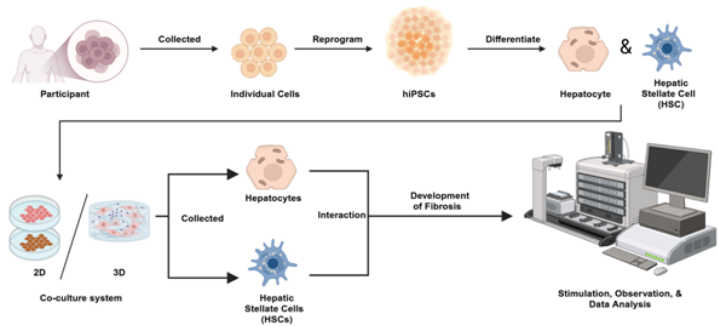

Figure 3. The involvement of human-induced pluripotent stem cells (hiPSCs) in the formulation of models for hepatic fibrosis

This reprogramming approach changes somatic cells into hiPSCs, which can later evolve into different liver cell types, including hepatocytes and hepatic stellate cells (HSCs). These cellular variants are then placed in co-culture across both two-dimensional (2D) and three-dimensional (3D) configurations to effectively imitate the fibrotic hepatic microenvironment.

5. 2D and 3D Cultures for Liver Fibrosis Modelling

Two-dimensional (2D) and three-dimensional (3D) culture systems are the two main techniques for mimicking liver fibrosis with iPSCs. Every method has distinct benefits that advance our knowledge of liver pathology and help create efficient treatment plans.

5.1 2D Culture Systems

The ability of iPSCs to imitate liver fibrosis in two-dimensional (2D) co-culture systems has emerged as a crucial field of study since it may help clarify the mechanisms behind liver fibrosis and pinpoint possible treatment targets. Since the interactions between hepatocytes and hepatic stellate cells are essential for the fibrogenic response, this method usually entails co-culturing those two. These technologies enable researchers to examine the dynamic interaction between hepatocytes and HSCs in

controlled settings. The relevance of intercellular communication in the evolution of fibrosis was highlighted by Royo et al (2013), who showed that extracellular vesicles generated by hepatocytes can affect HSC activation through the RNA they carry. Jones et al (2010) further underscored the important participation of hepatocytes in shaping the functions of hepatic stellate cells (HSC) regarding fibrosis, revealing that hepatocytes exposed to growth factors like hepatocyte growth factor (HGF) and bone morphogenetic protein 7 (BMP7) demonstrate defensive responses to injuries triggered by alcohol use.

Besides investigating disease processes, iPSC-derived hepatocyte-like cells (iPSC-HLCs) have demonstrated therapeutic efficacy in liver fibrosis models. Park et al (2019) emphasized that cells derived from iPSCs can mitigate liver fibrosis, highlighting their application in cell-based therapies intended to reverse fibrotic alterations. Enhancing the fidelity of these models, Suominen (2023) proposed the use of tissue-specific endothelial and mesenchymal cells in co-culture systems, which improves the differentiation and functionality of iPSC-HLCs. Cools (2024) revealed that co-culturing iPSC-derived hepatocytes with non-parenchymal cells promotes the development of intricate liver-like structures, hence enhancing the modeling of liver fibrosis.

Two-dimensional co-culture systems employing induced pluripotent stem cell-derived hepatocytes and hepatic stellate cells provide a robust platform for investigating liver fibrosis. These models enable an in-depth investigation of cellular interactions and provide a foundation for the development of novel treatment approaches for liver illnesses. Table 1 explores the diverse methodologies associated with 2D culture systems employed in the simulation of liver fibrosis utilizing iPSCs. However, despite their inherent simplicity and accessibility, two-dimensional co-culture systems are constrained by inadequate cell–cell and cell–matrix interactions, the degradation of tissue-specific architecture, and a lack of physiological relevance to the native hepatic environment [61], [62]. These constraints impede the precise modeling of fibrogenic pathways and pharmacological responses. Consequently, three-dimensional organoid-based models are progressively preferred owing to their ability to replicate both structural and functional characteristics of liver tissue, thereby facilitating more predictive outcomes in studies pertaining to fibrosis [63].

Table 1. Summary of 2D co-culture method in liver fibrosis modeling using induced pluripotent stem cells (iPSCs)

Cell Types	Key Findings	Methodology	Applications
iPSC-derived hepatocyte-like cells (iPSC-HLCs), endothelial cells (ECs), mesenchymal cells (Human iPSC) .	Early co-culture enhances differentiation and functionality of iPSC-HLCs [64].	2D co-culture with tissue-specific cells	Improved modeling of liver functionality

Cell Types	Key Findings	Methodology	Applications
Lgr5+ liver stem cells (Mouse), iPSC-derived cells (Human iPSC).	Induction of endogenous stem cells aids in liver repair [65].	Administration o soluble molecules	Potential for tissue damage repair
iPSC-derived hepatic endoderm, non-parenchymal cells (Human iPSC).	Mass production of liver buds from iPSCs [66].	Scalable culture platform	Development of transplantable liver tissues
iPSC-derived hepatocytes, hepatic stellate cells (Human iPSC).	Investigated mechanisms of liver fibrosis [67].	In vitro co-culture systems	Understanding liver fibrosis dynamics
iPSC-derived HSCs, hepatocyte-like cells (Human iPSC).	Differentiation of iPSCs to HSCs for fibrosis modeling [68].	2D and 3D culture models	Drug toxicity screening and fibrosis modeling
Quiescent HSC-like cells, hepatocytes (Human, derived from iPSC).	Development of quiescent HSCs for drug discovery [69].	Co-culture systems	Therapeutic target identification
iPSC-derived liver organoids (Human iPSC).	Organoids mimic liver functions and fibrosis [52].	Organoid culture	Modeling compound-induced liver diseases
iPSC-derived hepatocyte-like cells (Human iPSC).	Potential for hepatocyte transplantation [70].	Cell differentiation protocols	Cellular medicine applications
iPSC-derived hepatic endoderm, endothelial cells (Human iPSC) .	Enhanced hepatic maturation in liver assemblies [71].	Co-culture with endothelial cells	Therapeutic effects on liver fibrosis
iPSC-derived hepatocyte-like cells (Human iPSC).	Hepatocyte differentiation for liver therapy [72].	2D and 3D PEG-DA based scaffold culture models	Cellular therapy
iPSC-derived hepatocyte-like cells (Human iPSC).	Drug screening for hypercholesterolemia [73].	High-throughput screening	Identifying potential treatments for liver diseases

5.2 3D Culture Systems

The Use of three-dimensional (3D) liver organoids derived from human induced pluripotent stem cells (hiPSCs) represents a significant advancement in modeling liver fibrosis. These organoids mimic the native liver microenvironment by accurately recapitulating cellular architecture and interactions, providing valuable insight into fibrotic progression. The process begins with the differentiation of hiPSCs into hepatic progenitor cells through sequential exposure to specific signaling molecules, including Activin A, Wnt3a, HGF, and FGF2, followed by maturation using factors such as oncostatin M (OSM) and dexamethasone. These progenitors subsequently self-organize into structured liver-like tissues.

To enhance physiological relevance, organoids are composed of key hepatic cell types—including hepatocytes, cholangiocytes, hepatic stellate cells (HSCs), and endothelial cells—each derived from directed differentiation protocols. Hepatocyte-like cells express markers such as albumin, cytochrome P450 3A4 (CYP3A4), and hepatocyte nuclear factor 4 alpha (HNF4α), while HSCs are identified by α-SMA, GFAP, and collagen I, reflecting their fibrogenic activity. The inclusion of these multiple lineages enables robust modeling of intercellular crosstalk, such as TGF-β-mediated HSC activation and collagen deposition [74]. Additionally, liver ductal organoids have been shown to reconstruct intrahepatic biliary structures, further supporting microenvironmental fidelity [75]. These systems enable disease-specific modeling under controlled conditions, such as TGF-β1-induced fibrotic remodeling [76], and simulate mechanical stiffness associated with fibrosis when cultured in hydrogels [77]. iPSC-derived liver organoids also offer applications in regenerative therapy, high-throughput drug screening, and personalized medicine [78]. Table 2 outlines the various approaches to simulate liver fibrosis using 3D liver organoids derived from hiPSCs. Despite these advantages, several limitations remain. The key strengths of liver organoids include:

- **Structural fidelity**: Reconstruct 3D liver-like architecture with metabolic zonation and cellular organization that resemble in vivo liver. [79], [80].
- **Multi-lineage integration**: Include hepatocytes, cholangiocytes, endothelial cells, and HSCs, enabling intercellular signaling studies relevant to fibrosis. [74], [81]
- **Functional relevance**: Exhibit partial liver-specific functions such as albumin secretion, drug metabolism, and response to hormonal cues. [82], [83]
- **Research utility**: Provide platforms for antifibrotic drug testing, disease modeling, and development of patient-specific therapies [84].

However, the following drawbacks hinder broader application:

- **Size limitations**: Restricted growth due to oxygen/nutrient diffusion, leading to necrotic cores in large constructs [85], [86].
- **Immature phenotype**: Retention of fetal markers like alpha-fetoprotein (AFP), indicating incomplete hepatocyte maturation [87].
- **Cellular imbalance**: Difficulty maintaining stable ratios and viability of specific hepatic cell types over time [83].

- **Batch-to-batch variability**: Differences in differentiation and structure across experiments reduce reproducibility [88].
- **Operational constraints**: High production costs, protocol complexity, and limited scalability for high-throughput applications [89], [90].
- **Lack of standardization**: Absence of unified protocols limits clinical translation and cross-study comparison [91].

Table 2. Overview of 3D liver organoid approaches for modeling liver fibrosis using induced pluripotent stem cells (iPSCs)

Cell Types	Key Findings	Methodology	Applications
Primary liver cells (Human/Animal), iPSCs (Human iPSC)	Organoids maintain histological and functional features of native liver tissue [92].	3D organoid culture	Disease modeling and drug screening
HepaRG (Human), THP-1 (Human), hTERT-HSC (Human)	3D co-culture mimics fibrotic phenotypes and interactions between cell types [93].	3D multicellular co-culture	Mechanistic studies on liver fibrosis
iPSC-derived hepatocytes (Human iPSC), Kupffer cells (Human/Animal)	Kupffer cells play a pivotal role in initiating fibrogenesis [94].	3D bioprinted liver model	Investigating immune-mediated fibrotic injury
Mesenchymal stem cells (Human/Animal)	3D spheroid culture enhances secretion of antifibrotic factors [95].	Spheroid culture	Therapeutic strategies for hepatic fibrosis
hESC exosomes (Human ESC), hepatic stellate cells (Human/Animal)	Exosomes reduce liver fibrosis via TGFβ pathway modulation [4].	3D culture with exosome treatment	Development of antifibrotic therapies
Placenta-derived mesenchymal stem cells (Human Placenta)	Extracellular vesicles derived from stem cells alleviate fibrotic conditions [76].	3D organoid culture	Exploring miRNA roles in fibrosis
iPSC-derived liver organoids (Human iPSC)	Hydrogel stiffness influences cellular behavior and fibrosis progression [77].	Mechano-modulatory hydrogels	Understanding mechanical influences on fibrotic development

Cell Types	Key Findings	Methodology	Applications
Mesenchymal stem cells (Human/Animal)	3D culture enhances therapeutic efficacy of mesenchymal stem cells [96].	3D mesenchymal stem cell culture	Potential treatments for liver cirrhosis
Human hepatic stellate cells (Human)	Novel 3D co-culture system enhances fibrosis modeling [97].	Co-culture with hepatic stellate cells	Studying liver disease mechanisms
iPSC-derived liver cells (Human iPSC)	3D co-culture exhibits characteristics of steatosis and fibrosis [98].	Co-culture model	Modeling nonalcoholic steatohepatitis (NASH)

6. Parenchymal Cells (PCs): hiPSC-Derived Hepatocytes

Human induced pluripotent stem cells (hiPSCs) can be differentiated into hepatocyte-like cells through a stepwise process involving definitive endoderm induction, hepatic specification, and maturation [99]. This is achieved using sequential exposure to signaling molecules such as Activin A, Wnt3a, HGF, FGF2, oncostatin M (OSM), and dexamethasone to guide lineage commitment and promote hepatic functionality [100]. The resulting cells express key hepatic markers including albumin, cytochrome P450 isoforms (e.g., CYP3A4, cytochrome P450 2D6 (CYP2D6)), and hepatocyte nuclear factor 4 alpha (HNF4α), which are indicative of metabolic, synthetic, and detoxification capacities [101], [102], [103]. Figure 4 summarizes this directed differentiation process, highlighting each developmental stage and the associated signaling molecules used to induce hepatic lineage commitment.

Functional assessments such as albumin secretion, bile acid production, and drug metabolism assays confirm hepatocyte-like functionality [104], [105], [106]. These hiPSC-derived hepatocytes serve as critical components in liver fibrosis modeling, enabling the study of hepatocyte-stellate cell interactions and providing a reliable platform for screening antifibrotic agents.

The process results in hepatocyte-like cells expressing key functional markers relevant for liver disease modeling.

7. Non-Parenchymal Cells (NPCs): hiPSC-Derived Macrophages

Human induced pluripotent stem cells (hiPSCs) can be differentiated into macrophages through the formation of embryoid bodies (EBs) and hematopoietic progenitor cells (HPCs), which subsequently develop into monocytes and mature macrophages [107], [108]. These hiPSC-derived macrophages can be polarized into M1 (pro-inflammatory) or M2 (anti-inflammatory) phenotypes using cytokines such as IFN-γ/LPS and IL-4, respectively [109]. Marker-based characterization distinguishes M1 macrophages by CD80, CD86, and CD64 expression, while M2 macrophages typically express CD206, CD204, and arginase-I [110], [111].

Functional assays—including phagocytosis tests, cytokine quantification (e.g.,

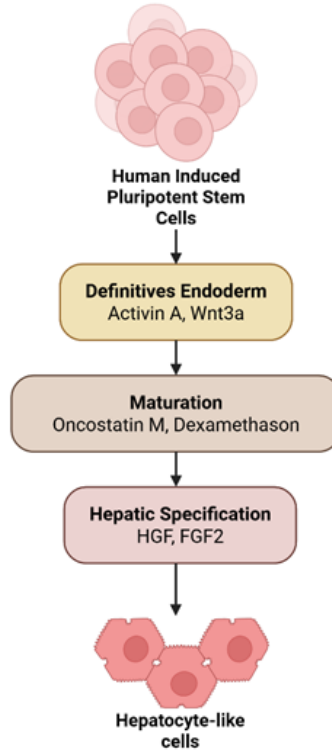

Figure 4. Directed differentiation of hiPSCs into hepatocyte-like cells via sequential exposure to Activin A, Wnt3a (definitive endoderm), HGF, FGF2 (hepatic specification), and OSM, dexamethasone (maturation)

TNF-α, IL-6), and nitric oxide measurements—are employed to assess macrophage activity [6], [112], [113]. These hiPSC-derived macrophages provide a relevant in vitro system to explore immune responses and inflammatory mechanisms in liver fibrosis, particularly in modeling macrophage–stellate cell interactions and evaluating immunomodulatory therapies.

8. Non-Parenchymal Cells (NPCs): hiPSCs-Derived Hepatic Stellate Cells

Hepatic stellate cells (HSCs) are non-parenchymal liver cells essential for ECM homeostasis and fibrosis development. The differentiation of human induced pluripotent stem cells (hiPSCs) into HSC-like cells typically involves three stages: mesoderm induction using Activin A and BMP4, hepatic specification through FGF2 and HGF, and maturation with TGF-β and ECM components to induce fibrogenic traits [114].

hiPSC-derived HSCs are identified by markers such as α-smooth muscle actin (α-SMA), glial fibrillary acidic protein (GFAP), and collagen type I—indicative of activation and fibrotic potential Marker expression is commonly evaluated through quantitative polymerase chain reaction (qPCR), immunohistochemistry, and Western blot [115], [116], [117].

Functional characterization includes collagen deposition assays (e.g., hydroxyproline content, Masson's trichrome staining) and responsiveness to profibrotic stimuli such as TGF-β, which promotes upregulation of α-SMA and collagen genes [118]. Co-culture models with hepatocytes or profibrotic cytokine treatment offer in vitro platforms to study HSC activation within a fibrotic microenvironment [119].

The ability to generate functional HSCs from hiPSCs provides a valuable tool for liver fibrosis modeling. These models enable mechanistic studies of fibrogenesis, cellular crosstalk, and antifibrotic drug screening, thus contributing to the development of precision therapies for chronic liver diseases.

9. Non-Parenchymal Cells (NPCs): hiPSCs-Derived Endothelial Cells

Human induced pluripotent stem cells (hiPSCs) offer a promising source for generating endothelial cells to support vascularized in vitro liver models. Differentiation of hiPSCs into endothelial cells mimics embryonic development—beginning with mesoderm induction and culminating in endothelial commitment via VEGF signaling and transcription factors such as ETV2 [120].The resulting cells express endothelial markers (e.g., CD31, VE-cadherin, vWF) and can form tube-like vascular structures [121]. In the liver context, hiPSC-derived endothelial cells contribute to sinusoid-like architecture by interacting with hepatocytes and non-parenchymal cells, thus enhancing extracellular matrix formation and cell signaling [51, p. 201]. Advances in differentiation protocols aim to improve the efficiency and functional relevance of these cells, particularly for liver fibrosis modeling where endothelial-stellate-hepatocyte interactions are pivotal. Representative methods for differentiating hiPSCs into liver-specific endothelial cells are summarized in Table 3.

Table 3. The list of established methodologies for differentiating hiPSCs into liver-specific endothelial cells

Aspect	Details
Advanced Programming Techniques	The use of the transcription factor ETV2 in transgenic hiPSCs enables rapid and cost-effective generation of endothelial cells (ECs) without requiring intermediate purification steps. This method produces functional ECs exhibiting characteristics such as tube formation and LDL uptake, essential for disease modeling and tissue engineering applications [122].
Liver-Specific Endothelial Cell Differentiation	hiPSC-derived endothelial cells can differentiate into liver sinusoidal endothelial cells (LSECs) following transplantation into mouse livers. This process is characterized by the expression of human factor VIII and other LSEC markers. Transcriptomic analysis reveals that transcription factors such as NOTCH1 and GATA4 are crucial for this specification, indicating a complex regulatory network involved in LSEC maturation [123].

Co-Differentiation with Hepatocytes	Co-differentiation protocols incorporating BMP4 enhance the maturation of hiPSC-derived hepatocytes by including NPCs, which play a critical role in liver development. This approach improves the functionality of hepatocyte-like cells and highlights the importance of NPCs in liver organogenesis [124].

The identification of precise molecular indicators is critical for elucidating the successful differentiation of human induced pluripotent stem cell-derived endothelial cells. These indicators signify the attainment of endothelial identity and functional maturation. Among these markers is CD31, which is frequently used to identify endothelial cells because of its unique expression and function in promoting intercellular adhesion [122]. One transmembrane protein that indicates the development of endothelial cell junctions is called VE-cadherin [123]. Additionally, endothelial cells emit a protein called von Willebrand Factor (vWF), which is deposited in eibel-Palade bodies. This protein functions as a functional marker that shows how well endothelial cells can react to a thrombogenic stimulus [124].

Understanding vascular biology and creating treatment plans depend on functional assays that quantify endothelial permeability, migration, and angiogenesis in non-parenchymal cells (NPCs), especially endothelial cells produced from human-induced pluripotent stem cells (hiPSCs). The functional ability of hiPSC-derived endothelial cells (hiPSC-ECs), which are becoming more well-known for their promise in regenerative medicine, is revealed by these assays. Table 4 summarizes key functional assays employed to evaluate the physiological properties of hiPSC-derived endothelial cells (hiPSC-ECs), including their barrier integrity, migratory behavior, and angiogenic capacity in both 2D and 3D in vitro models.

Table 4. Functional assays to provide functional capacity of iPSC-EC

Functional Assay	Description
Real-Time Impedance Spectroscopy	Measures electrical resistance across an endothelial monolayer, providing insights into permeability changes during inflammatory responses [125].
Fluorescent Tracer Studies	Uses fluorescent dyes in 3D microvascular models to evaluate permeability during angiogenic sprouting [126].
Wound Healing Assay	A 2D assay that measures the migration of endothelial cells into a wound area, simulating tissue repair [127].
Zebrafish Xenograft Model	Allows assessment of hiPSC-EC migration and integration into host tissues, requiring a small number of cells [120].
Spheroid Sprouting Assay	A 3D assay that evaluates endothelial sprouting from spheroids in a collagen matrix, mimicking in vivo angiogenesis.

Functional Assay	Description
Perfused 3D Microvessel Model	Studies angiogenic processes under physiological conditions, including lumen formation and differentiation into tip and stalk cells [126].

10. Induction of Liver Fibrosis in Liver Organoid

The establishment of liver fibrosis models utilizing organoid systems has yielded significant revelations concerning the pathophysiology of liver disorders and the advancement of therapeutic interventions. Research regarding Non-Alcoholic Steatohepatitis (NASH) suggests that palmitic acid exposure can convincingly mimic a disease-like condition in human individuals. Ouchi and associates (2019) illustrated that the introduction of palmitic acid (0.25-0.5 mM) over a timeframe of 24-48 hours in liver organoids led to significant lipid buildup within the cells, which subsequently activated the endoplasmic reticulum stress signaling and raised the levels of reactive oxygen species (ROS). Such scenarios give rise to an inflammatory cascade, noted for an increased expression of proinflammatory cytokines, which include TNF-α, IL-6, and IL-1β. Furthermore, de l'Hortet et al (2019) illustrated that prolonged exposure to palmitic acid culminated in the activation of hepatic stellate cells, as evidenced by elevated levels of α-SMA and the synthesis of extracellular matrix proteins, particularly collagen types I and III. Complementary single-cell RNA sequencing corroborated transcriptional modifications that align with the disease profile of human NASH, encompassing the upregulation of genes pertinent to lipid metabolism and inflammatory responses. The application of acetaminophen (acetaminophen (APAP)) in the liver organoid model yielded significant insights into the mechanisms underlying drug-induced liver injury. Rashidi et al (2018) demonstrated that exposure to APAP (5-10 mM) triggers a series of molecular events, starting with glutathione depletion and leading to the production of the reactive metabolite N-acetyl-p-benzoquinone imine (N-acetyl-p-benzoquinone imine (NAPQI)). This process induces significant oxidative stress, leads to mitochondrial dysfunction, and ultimately results in hepatocyte necrosis. Prill et al (2016) found that APAP-induced damage to liver organoids activates a regenerative response characterized by progenitor cell proliferation and matrix remodeling. Hu et al (2018) characterized the transcriptomic changes during this process, identifying the activation of signaling pathways, including Hippo/YAP and Notch, which are crucial for post-injury liver regeneration.

Both models demonstrated significant characteristics of fibrosis progression, including the activation of hepatic stellate cells, excessive deposition of extracellular matrix proteins, and disruption of normal tissue architecture. The elevation of TGF-β, PDGF, and other profibrotic mediators was associated with the severity of observed fibrosis [66]. The application of single-cell RNA sequencing and proteomics in these models has elucidated the heterogeneity of cell populations and molecular pathways associated with fibrosis progression, offering valuable insights for the advancement of targeted antifibrotic therapies.

11. Future Direction

In addition to advancements in biomaterials and 3D bioprinting, future research in hiPSC-derived liver organoids is expanding toward system-level integration and personalized medicine. A key direction involves the development of multi-organ models—such as gut–liver or liver–pancreas platforms—to replicate inter-organ communication that influences metabolic regulation and fibrosis progression [128], [129]. Vascularization strategies, including co-culture with hiPSC-derived endothelial cells and integration into perfusable microfluidic systems, are being explored to recreate liver-specific sinusoidal networks, thereby improving tissue perfusion and physiological relevance [53], [83].

The creation of patient-specific organoids is also gaining attention. These models allow for personalized investigation of fibrotic pathways and drug responses based on a patient's genetic or epigenetic background [130] . Alongside structural innovations, omics integration—such as transcriptomic and proteomic profiling—can provide detailed insight into disease mechanisms [131]. Combining these approaches with machine learning may help uncover predictive markers of fibrosis and refine antifibrotic drug discovery pipelines [131].

Emerging technologies like 4D bioprinting offer dynamic platforms where stimuli-responsive materials mimic real-time tissue changes [132]. This can enhance the accuracy of fibrosis modeling, especially for studying tissue remodeling. While these innovations are promising, challenges such as variability between batches, incomplete cell maturation, and regulatory complexities remain significant [132]. Addressing these issues is essential to maximize the translational potential of hiPSC-derived liver organoids in disease modeling and therapeutic development [133].

12. Conclusion

Human induced pluripotent stem cell (hiPSC)-derived liver organoids represent a transformative platform for modeling liver fibrosis, offering unprecedented opportunities to investigate fibrogenic mechanisms, evaluate antifibrotic agents, and develop patient-specific therapies. These models recapitulate the complex architecture and multicellular interactions of the native liver, enabling a more accurate simulation of fibrotic processes compared to traditional in vitro systems. Through directed differentiation, hiPSCs can give rise to hepatocytes, hepatic stellate cells, endothelial cells, and macrophages—cell types essential for mimicking the cellular and molecular dynamics of liver fibrosis.

While current therapeutic strategies for liver fibrosis are often constrained by limited efficacy, long treatment durations, and the invasiveness of procedures such as liver biopsy, hiPSC-based systems present a promising alternative. In particular, three-dimensional organoid cultures offer enhanced physiological relevance and potential for high-throughput drug screening and regenerative applications. Despite the promise, challenges remain—including variability between batches, incomplete cellular maturation, and the need for vascularization and immune integration.

Future directions focused on multi-organ modeling, incorporation of microfluidic systems, and integration with omics and machine learning approaches will likely accelerate the translational application of these models. Addressing current limitations

through interdisciplinary innovation is essential to fully realize the potential of hiPSC-derived liver organoids as a robust, scalable, and ethically viable platform for advancing liver fibrosis research and the development of effective, targeted antifibrotic therapies.

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