Generation of Liver Organoids

Subjects: Cell Biology

Contributor: Maria G. Roubelakis, Christina Nikokiraki

The liver represents the most important metabolic organ of the human body. It is evident that an imbalance of liver function can lead to several pathological conditions, known as liver failure. Orthotropic liver transplantation (OLT) is currently the most effective and established treatment for end-stage liver diseases and acute liver failure (ALF). Due to several limitations, stem-cell-based therapies are currently being developed as alternative solutions. Stem cells or progenitor cells derived from various sources have emerged as an alternative source of hepatic regeneration. Therefore, hematopoietic stem cells (HSCs), mesenchymal stromal cells (MSCs), endothelial progenitor cells (EPCs), embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are also known to differentiate into hepatocyte-like cells (HPLCs) and liver progenitor cells (LPCs) that can be used in preclinical or clinical studies of liver disease. Furthermore, these cells have been shown to be effective in the development of liver organoids that can be used for disease modeling, drug testing and regenerative medicine.

Keywords: liver disease ; clinical trials ; organoids ; EC ; iPSCs

1. Introduction

Organoids represent a novel approach in the frame of liver regenerative medicine that could be utilized as transplantable units, disease models, toxicological studies or tools for drug discovery. Organoids, which are 3D self-organized scaffolds based on cellular structures that are derived from differentiated ESCs, iPSCs or adult stem cells (ASCs), are grown in a serum-free condition and display the full spectrum of cellular types in a tissue [1]. More specifically, a liver organoid is a 3D multicellular spherical structure made of one or more liver cell types [2]. Brassard et al. developed some parameters in order to control the process of building a 3D organoid [3]. Importantly, it has been suggested that the choice and control of the number of cells and the density of the dissociation–aggregation phase is mandatory, including the best culture conditions and the combination of growth factors to specifically guide the differentiation process. In liver organoids, the molecular signaling pathways that regulate liver embryonic development, such as HGF, FGF, BMP, Wnt and TGF, could guide the growth factor supplementation, which promotes hepatic progenitor migration, development and survival [4]. Furthermore, in order to build a 3D organoid, it is required to supply (if necessary) some pre-defined extrinsic forces to improve cell-to-cell and cell-to-extracellular-matrix (ECM) interactions [3]. Because the ECM plays a crucial role in supporting cell proliferation, improving cell adherence and the dispersal of nutrients and growth factors, stem/progenitor cells must be in a strict contact with its components, such as collagen, laminin and fibronectin [5]. Finally, micropatterning, microwells and microfluid dynamics are some of the designed geometries that can be utilized to support cell and organoid growth [3].

Liver organoid technology simulates the morphological and physiological properties and tissue-specific functions of the liver in a dish through the self-organization of cell populations that mimic the liver development process [6]. Liver organoids could be derived either from the culture of a single cell type, such as adult stem cells, LPCs or iPSCs, or from a multi-type cell co-culture, such as:

- A combination of iPSCs with human umbilical vein endothelial cells (HUVECs) and MSCs;
- Hepatocytes and stromal cells;
- Primary liver tumor cells or damaged liver cells [7].

The single type of cell culture ensures the proliferation and self-organization of a homogenous cell population and is easier to form, while co-culture of multiple cells types can better mimic the liver organ structure [8][9].

2. Single Cell-Type Culture for Organoid Development

The first single cell-type culture of a human ASC-derived organoid was developed by culturing $LGR5^+$ biliary cells derived from a liver injury by adding Rspo1, EGF, FGF10, HGF, nicotinamide, cAMP agonist and TGFβ receptor inhibitor to the differentiation medium [10]. Since then, other large-scale culturing methods for ASC-derived liver organoids have been established [11]. A number of studies have already proposed the development of single-cell culture-derived liver organoids by the use of liver progenitor cells. First, Hu et al. embedded human mature primary hepatocytes in Matrigel to form liver organoids using molecule inducers, such as Rspo1, EGF, FGF7, FGF10, HGF and TGFβ inhibitor [12]. As a result, they developed human fetal hepatocyte organoids (Hep-Orgs), which were cultured for more than 11 months [12]. After that, in 2020, Hendriks et al. established a protocol with similar culture conditions that facilitate the long-term expansion of human fetal hepatocytes as organoids. [13].

Furthermore, the use of iPSC-derived liver organoids has already been proposed for the treatment of liver diseases. Because liver progenitors arise from the endoderm, iPSCs must first be differentiated into definitive endoderm to mimic liver development [14] and further exhibit hepatic maturation in alternate normoxia and hypoxia conditions [15]. Following that, liver organoids will be formed over 2D monolayers of mature hepatocytes that will then be collected and embedded on Matrigel [15]. In 2022, Messina et al. generated human iPSC-derived hepatocytes (iHeps) that self-assembled as organoids (iHep-Orgs) [15]. In particular, human iPSCs were first differentiated into hepatoblasts (iHBs) using growth factors and cytokines according to other already published protocols [15][16]. Then, iHBs were developed into iHep-Orgs using HGF, vitamin K, OSM and Dex, and their culture extended until day 38, as they were still viable, with the absence of necrotic cores [15]. Their successful development of those iHep-Orgs was then confirmed by several studies, including gene expression for *EPCAM* and *CXCR4*, the absence of pluripotent markers and the presence of hepatoblast markers as well as by the well-defined morphology [1][7][13][17][18][19][20].

3. Multi-Type Cell Co-Culture for Organoid Development

To ensure cell-to-cell contacts during liver development, multi-cell co-cultures are able to generate a complex vascularized 3D structure in dishes [21]. In 2013, Takebe et al. generated, for the first time, a vascularized and functional human liver from human iPSCs by the transplantation of liver buds that were created in vitro [22]. More specifically, iPSCs were co-cultured with HUVECs and MSCs and spontaneously self-organized into macroscopically visible 3D cell aggregates showing endothelial network and the expression of hepatic-specific marker genes [22]. After that, the whole liver buds were connected to the recipient's vasculature to generate functional vascular networks, promoting liver bud development [22]. Since MSCs can provide a number of signals promoting hepatocyte growth and development, co-culture of BM-MSCs and hepatocytes exhibited higher rates of hepatocyte-specific functions, maintained hepatocyte metabolism and generated higher quality hepatocyte organoids [23]. Although some studies suggested that MSC and HUVEC paracrine signals can both drive hepatocyte differentiation, both must co-exist to allow for cell-to-cell interaction and organization into a 3D liver organoid [24]. In 2018, Nie et al., successfully generated human liver organoids derived from multiple cells from a single donor, and these organoids were finally able to rescue a mouse model of ALF [25]. In more detail, umbilical cord endothelial cells (UC-ECs) were able to generate donor-derived human iPSCs, which then were able to efficiently differentiate into pure definitive endoderm and further into the hepatic lineage, and with a co-culture of these three cell types (UC-ECs, iPSCs and MSCs), the investigators successfully generated a single-donor cell-derived liver organoid [25]. In 2021, Qiu et al. generated a functional 3D sheet-like human hepatocellular carcinoma (HCC) organoid in vitro by co-culturing luciferase-expressing Huf7 cells, hiPSCs-derived endothelial cells and hiPSC-derived MSCs [26]. According to their results, once they added iPSC-MSCs, the organization of the 3D HCC organoid was easier, suggesting that iPSC-MSCs play a crucial role in promoting HCC organoid growth [26].

Although iPSCs appear to be beneficial in 3D liver organoid development, some studies tested a co-culture of MSCs and HUVECS with other cell types, such as amniotic stem cells (ASCs), resulting in the generation of a 3D structure with polarity and hepatic-like glycogen storage [27]. Co-culturing methods of human hepatocytes, HUVECS and MSCs have also been applied [28]. Moreover, the privilege of co-culturing human hepatocytes, HUVECs and MSCs has been tested in fusing hundreds of liver-bud-like spheroids using a 3D bioprinter [29]. Currently, studies for organoid development focus on co-culturing mesenchymal and endothelial cells, as these cells are known for their ability to regulate liver progenitor cells' fate and growth [30].

Furthermore, the co-culturing of primary liver tumor [31][32][33] or damaged liver cells [6][34] has also been studied. Patient-derived tumor organoids (PDO) are 3D cell culture models that closely mimic the form and function of tumor tissue, illustrate cell-to-cell and cell-to-matrix interactions and have similar pathophysiological characteristics to differentiated tumor tissue in vitro [21]. Primary liver tumor organoids can be derived from liver tumor specimens, preserving the

histological architecture, gene expression and genomic landscape of the original tumor, providing a tool for biomarker identification and drug screening [32]. Liver organoids can also be derived from patient biopsies with alpha-1-antitrypsin (ATT) syndrome, which is a useful tool for modeling disease pathology [7]. In addition, in 2021, McCarron et al. received wedge biopsies from patients with nonalcoholic steatohepatitis (NASH) and developed hepatic organoids, suggesting that these organoids can be used for personalized disease modeling and drug development [34]. Consequently, liver organoids can be derived from either healthy or injured patient tissues. Genetic modifications of these organoids enable disease modeling, the clarification of molecular pathogenesis, patient-specific drug testing, personalized medicine and biomarker discovery, causing them to be an alternative model for biomedical research [35][36].

References

1. Messina, A.; Luce, E.; Hussein, M.; Dubart-Kupperschmitt, A. Pluripotent-Stem-Cell-Derived Hepatic Cells: Hepatocytes and Organoids for Liver Therapy and Regeneration. Cells 2020, 9, 420.
2. Chang, M.; Bogacheva, M.S.; Lou, Y.-R. Challenges for the Applications of Human Pluripotent Stem Cell-Derived Liver Organoids. Front. Cell Dev. Biol. 2021, 9, 2715.
3. Brassard, J.A.; Lutolf, M.P. Engineering Stem Cell Self-organization to Build Better Organoids. Cell Stem Cell 2019, 24, 860–876.
4. Gamboa, C.M.; Wang, Y.; Xu, H.; Kalemba, K.; Wondisford, F.E.; Sabaawy, H.E. Optimized 3D Culture of Hepatic Cells for Liver Organoid Metabolic Assays. Cells 2021, 10, 3280.
5. Hsia, G.S.P.; Esposito, J.; da Rocha, L.A.; Ramos, S.L.G.; Okamoto, O.K. Clinical Application of Human Induced Pluripotent Stem Cell-Derived Organoids as an Alternative to Organ Transplantation. Stem Cells Int. 2021, 6632160.
6. Akbari, S.; Arslan, N.; Senturk, S.; Erdal, E. Next-Generation Liver Medicine Using Organoid Models. Front. Cell Dev. Biol. 2019, 7, 345.
7. Luo, Y.; Zhang, M.; Chen, Y.; Chen, Y.; Zhu, D. Application of Human Induced Pluripotent Stem Cell-Derived Cellular and Organoid Models for COVID-19 Research. Front. Cell Dev. Biol. 2021, 9, 720099.
8. Green, C.J.; Charlton, C.A.; Wang, L.-M.; Silva, M.; Morten, K.J.; Hodson, L. The isolation of primary hepatocytes from human tissue: Optimising the use of small non-encapsulated liver resection surplus. Cell Tissue Bank. 2017, 18, 597–604.
9. Du, Y.; Wang, J.; Jia, J.; Song, N.; Xiang, C.; Xu, J.; Hou, Z.; Su, X.; Liu, B.; Jiang, T.; et al. Human Hepatocytes with Drug Metabolic Function Induced from Fibroblasts by Lineage Reprogramming. Cell Stem Cell 2014, 14, 394–403.
10. Gómez-Mariano, G.; Matamala, N.; Martínez, S.; Justo, I.; Marcacuzco, A.; Jimenez, C.; Monzón, S.; Cuesta, I.; Garfia, C.; Martínez, M.T.; et al. Liver organoids reproduce alpha-1 antitrypsin deficiency-related liver disease. Hepatol. Int. 2020, 14, 127–137.
11. Schneeberger, K.; Sánchez-Romero, N.; Ye, S.; van Steenbeek, F.G.; Oosterhoff, L.A.; Palacin, I.P.; Chen, C.; van Wolferen, M.E.; van Tienderen, G.; Lieshout, R.; et al. Large-Scale Production of LGR5-Positive Bipotential Human Liver Stem Cells. Hepatology 2020, 72, 257–270.
12. Hu, H.; Gehart, H.; Artegiani, B.; Löpez-Iglesias, C.; Dekkers, F.; Basak, O.; Van Es, J.; Chuva de Sousa Lopes, S.M.; Begthel, H.; Korving, J.; et al. Long-Term Expansion of Functional Mouse and Human Hepatocytes as 3D Organoids. Cell 2018, 175, 1591–1606.e19.
13. Hendriks, D.; Artegiani, B.; Hu, H.; Lopes, S.C.D.S.; Clevers, H. Establishment of human fetal hepatocyte organoids and CRISPR–Cas9-based gene knockin and knockout in organoid cultures from human liver. Nat. Protoc. 2021, 16, 182–217.
14. Kulkeaw, K.; Tubsuwan, A.; Tongkrajang, N.; Whangviboonkij, N. Generation of human liver organoids from pluripotent stem cell-derived hepatic endoderms. PeerJ 2020, 8, e9968.
15. Messina, A.; Luce, E.; Benzoubir, N.; Pasqua, M.; Pereira, U.; Humbert, L.; Eguether, T.; Rainteau, D.; Duclos-Vallée, J.-C.; Legallais, C.; et al. Evidence of Adult Features and Functions of Hepatocytes Differentiated from Human Induced Pluripotent Stem Cells and Self-Organized as Organoids. Cells 2022, 11, 537.
16. Caron, J.; Pène, V.; Tolosa, L.; Villaret, M.; Luce, E.; Fourrier, A.; Heslan, J.-M.; Saheb, S.; Bruckert, E.; Gómez-Lechón, M.J.; et al. Low-density lipoprotein receptor-deficient hepatocytes differentiated from induced pluripotent stem cells allow familial hypercholesterolemia modeling, CRISPR/Cas-mediated genetic correction, and productive hepatitis C virus infection. Stem Cell Res. Ther. 2019, 10, 221.

17. Dianat, N.; Dubois-Pot-Schneider, H.; Steichen, C.; Desterke, C.; Leclerc, P.; Raveux, A.; Combettes, L.; Weber, A.; Co rlu, A.; Dubart-Kupperschmitt, A. Generation of functional cholangiocyte-like cells from human pluripotent stem cells an d HepaRG cells. Hepatology 2014, 60, 700–714.
18. Tao, T.; Deng, P.; Wang, Y.; Zhang, X.; Guo, Y.; Chen, W.; Qin, J. Microengineered Multi-Organoid System from hiPSCs to Recapitulate Human Liver-Islet Axis in Normal and Type 2 Diabetes. Adv. Sci. 2021, 9, 2103495.
19. Gurevich, I.; Burton, S.A.; Munn, C.; Ohshima, M.; Goedland, M.E.; Czysz, K.; Rajesh, D. iPSC-derived hepatocytes ge nerated from NASH donors provide a valuable platform for disease modeling and drug discovery. Biol. Open 2020, 9, bi o055087.
20. Nguyen, R.; Da Won Bae, S.; Qiao, L.; George, J. Developing Liver Organoids from Induced Pluripotent Stem Cells (IP SCs): An Alternative Source of Organoid Generation for Liver Cancer Research. Cancer Lett. 2021, 508, 13–17.
21. Zhu, X.; Zhang, B.; He, Y.; Bao, J. Liver Organoids: Formation Strategies and Biomedical Applications. Tissue Eng. Re gen. Med. 2021, 18, 573–585.
22. Takebe, T.; Sekine, K.; Enomura, M.; Koike, H.; Kimura, M.; Ogaeri, T.; Zhang, R.; Ueno, Y.; Zheng, Y.-W.; Koike, N.; et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 2013, 499, 481–484.
23. He, J.; Zhang, X.; Xia, X.; Han, M.; Li, F.; Li, C.; Li, Y.; Gao, D. Organoid technology for tissue engineering. J. Mol. Cell Biol. 2020, 12, 569–579.
24. Asai, A.; Aihara, E.; Watson, C.; Mourya, R.; Mizuochi, T.; Shivakumar, P.; Phelan, K.; Mayhew, C.; Helmrath, M.; Takeb e, T.; et al. Paracrine signals regulate human liver organoid maturation from Induced Pluripotent Stem Cells. Developm ent 2017, 144, 1056–1064.
25. Nie, Y.-Z.; Zheng, Y.-W.; Ogawa, M.; Miyagi, E.; Taniguchi, H. Human liver organoids generated with single donor-deriv ed multiple cells rescue mice from acute liver failure. Stem Cell Res. Ther. 2018, 9, 5.
26. Qiu, R.; Murata, S.; Cheng, C.; Mori, A.; Nie, Y.; Mikami, S.; Hasegawa, S.; Tadokoro, T.; Okamoto, S.; Taniguchi, H. A Novel Orthotopic Liver Cancer Model for Creating a Human-like Tumor Microenvironment. Cancers 2021, 13, 3997.
27. Furuya, K.; Zheng, Y.-W.; Sako, D.; Iwasaki, K.; Zheng, D.-X.; Ge, J.-Y.; Liu, L.-P.; Furuta, T.; Akimoto, K.; Yagi, H.; et a l. Enhanced hepatic differentiation in the subpopulation of human amniotic stem cells under 3D multicellular microenvir onment. World J. Stem Cells 2019, 11, 705–721.
28. Takebe, T.; Sekine, K.; Suzuki, Y.; Enomura, M.; Tanaka, S.; Ueno, Y.; Zheng, Y.-W.; Taniguchi, H. Self-Organization of Human Hepatic Organoid by Recapitulating Organogenesis In Vitro. In Transplantation Proceedings; Elsevier BV: Amst erdam, The Netherlands, 2012; Volume 44, pp. 1018–1020.
29. Yanagi, Y.; Nakayama, K.; Taguchi, T.; Enosawa, S.; Tamura, T.; Yoshimaru, K.; Matsuura, T.; Hayashida, M.; Kohashi, K.; Oda, Y.; et al. In vivo and ex vivo methods of growing a liver bud through tissue connection. Sci. Rep. 2017, 7, 1408 5.
30. Ober, E.A.; Lemaigre, F.P. Development of the liver: Insights into organ and tissue morphogenesis. J. Hepatol. 2018, 6 8, 1049–1062.
31. Saito, Y.; Muramatsu, T.; Kanai, Y.; Ojima, H.; Sukeda, A.; Hiraoka, N.; Arai, E.; Sugiyama, Y.; Matsuzaki, J.; Uchida, R.; et al. Establishment of Patient-Derived Organoids and Drug Screening for Biliary Tract Carcinoma. Cell Rep. 2019, 27, 1265–1276.e4.
32. Broutier, L.; Mastrogiovanni, G.; Verstegen, M.M.A.; Francies, H.E.; Gavarró, L.M.; Bradshaw, C.R.; Allen, G.E.; Arnes-Benito, R.; Sidorova, O.; Gaspersz, M.P.; et al. Human primary liver cancer–derived organoid cultures for disease mod eling and drug screening. Nat. Med. 2017, 23, 1424–1435.
33. Vlachogiannis, G.; Hedayat, S.; Vatsiou, A.; Jamin, Y.; Fernández-Mateos, J.; Khan, K.; Lampis, A.; Eason, K.; Huntingf ord, I.; Burke, R.; et al. Human Primary Liver Cancer Organoids Reveal Intratumor and Interpatient Drug Response Het erogeneity. JCI Insight 2019, 4, e121490.
34. McCarron, S.; Bathon, B.; Conlon, D.M.; Abbey, D.; Rader, D.J.; Gawronski, K.; Brown, C.D.; Olthoff, K.M.; Shaked, A.; Raabe, T.D. Functional Characterization of Organoids Derived From Irreversibly Damaged Liver of Patients With NAS H. Hepatology 2021, 74, 1825–1844.
35. Drost, J.; Clevers, H. Organoids in cancer research. Nat. Rev. Cancer 2018, 18, 407–418.
36. Rao, S.; Hossain, T.; Mahmoudi, T. 3D human liver organoids: An in vitro platform to investigate HBV infection, replicati on and liver tumorigenesis. Cancer Lett. 2021, 506, 35–44.