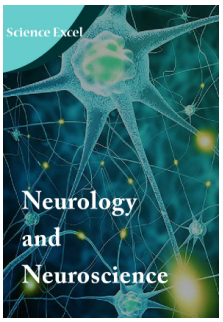
Neurology & Neuroscience

Review Article



Curing Classical Homocysteinuria with Pediatric *in vitro engineered* Liver Organoid Transplantation?

Stefan Bittmann, Elisabeth Luchter, Lara Bittmann, Elena Moschüring-Alieva, Gloria Villalon

Ped Mind Institute (PMI), Department of Pediatrics, Hindenburgring 4, D-48599 Gronau, Germany

*Correspondence

Stefan Bittmann, Visit. Prof., M.D., M.A. Ph.D.

Head of Ped Mind Institute

Visiting Professor (SVCT, Shangluo, China)
Ped Mind Institute (PMI), Department of Pediatrics Medical and Finance Center Epe
Gronau, Germany

Tel. +49-2565-97325

Fax. +49-2565-97324

- Received Date: 05 May 2023
- Accepted Date: 09 May 2023
- Publication Date: 13 May 2023

Abstract

Organoids are groups of cells grown in the laboratory that have self- organized into cell structures resembling those of organs. The term "organoid" means "organ-like." In many cases, the cells and cell structures give organoids abilities similar to those of the organs they resemble. Brain organoids, for example, develop layers of nerve cells (neurons) with signaling activity and even "brain regions" that resemble areas of the human brain. Currently, organoids created by researchers may bear a strong resemblance to a fully mature organ in certain respects, but crucial differences still exist. Intestinal organoids have a variety of cellular structures that resemble parts of the intestinal mucosa, but are typically the size of a pea and thus not nearly as large or complex as our intestinal tract. But even though they are small, or do not correspond one hundred percent to entire organs, scientists can learn a lot from organoids. Experts believe that organoids represent the "next generation" of biological tools for research, drug development and medicine. Liver Organoid engineering could play an important role in treating and curing classic homocysteinuria in childhood by resetting the disturbed function of cystathion beta synthetase activity in the implanted organoid liver. This manuscript focus on liver organoid research to date with special attention to classic homocysteinuria in childhood.

Introduction

An organoid is an organ-like microstructure a few millimeters in size that can be artificially generated using cell culture methods. Under appropriate culture conditions, organoids can be grown from one or a few tissue cells, embryonic stem cells, or induced pluripotent stem cells. Unless mesenchymal stem cells have been used, organoids lack stroma and vessels; however, they still exhibit physiologically relevant organ-like properties. The generation of organoids requires pluripotent stem cells as starting material. Such cells are in a state from which they are capable of differentiating and structuring themselves together. The result of self-organization is tissue-like associations of differentiated cells that differ in shape and function. The structure of organoids at least partially resembles human or animal organs. Organoids are usually not formed on an agar layer; they require liquid culture medium that provides the opportunity to grow spatially in a 3D cell culture. The production of organoids requires a sterile cell culture laboratory to perform the sophisticated, constructive tissue engineering. This field of work and research in biotechnology may also use genetic engineering techniques, most notably the CRISPR/Cas method. The product range includes tiny models of internal organs (heart, stomach, intestine, kidney).

Amazing progress is being made with the complex structures of the brain. Such cerebral organoids model cerebral cortex, hippocampus, midbrain, hypothalamus, cerebellum, anterior pituitary, and ocular retina of humans, mammals, and less commonly other vertebrates. Protocols exist for growing them, which cause regions of the brain to develop. Researchers are developing mini-livers, or liver replacement tissue, which temporarily improves the performance of the liver. This involves reprogramming skin cells in the laboratory with four substances, the Yamanaka factors, which convert them into stem cells. From these cells, all cell types of the body, including healthy liver cells, are grown. Another option is to use adult liver cells from human tissue. These liver cells come from patients who are biopsied with a large needle. A third option is to stimulate the small functional tissue areas from an otherwise old, diseased liver with growth factors or certain proteins so that the stem cells or adult cells develop tiny three-dimensional structures: Organoids made of liver cells, special immune cells, bile ducts and blood vessels. Stem cells can first be grown

Citation: Bittmann S, Luchter E, Bittmann L, Moschüring-Alieva E, Villalon G. Curing Classical Homocysteinuria with Pediatric in vitro engineered Liver Organoid Transplantation?. Neurol Neurosci. 2023;4(1):1-6.

from patients' skin cells, and then "miniature brains" or "mini-livers" can be grown from them, so-called "organoids". Stem cells can be grown from patients' skin cells and then used to grow "miniature brains" or "miniature livers", so-called "organoids". Researchers have transplanted mini-livers into sick mice. They grew amazingly well and took over the function of the damaged organ. The next step is to implant the liver organoids into the livers of deceased humans. In Vitro Engineering of Organoids is based on different steps. Step 1 is preparing a 2D preculture.

Organoids are generated from either primary cells (i.e. intestine, lung, or kidney) or induced pluripotent stem cells. Stem cells are capable of differentiating and self-assembling into a variety of specific organoids. Typically, cells are mixed with Matrigel and drops of this mixture are placed in a 24-well plate at room temperature.

The plates are then placed in an incubator to form solid droplet domes. Media is then added for seven or more days to promote cell growth and differentiation into a specific tissue (brain, intestine, lung, etc.). The media contains extracellular matrix (ECM) proteins and various growth factors that vary depending on which tissue is being generated. Organoid culture is a lengthy process and may involve several cultivation steps with different media. During this process, the health of the cells must be monitored using methods normally used to track developmental biology and understand tissues (imaging). Before experiments are performed, organoids must be reviewed and characterized to ensure they have the correct tissue structure and differentiation. High-content imaging allows for verification and visualization of organoid growth and differentiation, 3D reconstruction of structures, complex analyses of organoid structure, cell morphology and survival, and expression of various cell markers. Confocal imaging and 3D analysis of organoids allow visualization and quantification of the organoids and the cells that form the organoid. Characterization of multiple quantitative descriptive features of organoids can be applied to explore disease phenotypes and connectivity effects.

Types of Organoids (Epithelial/Multi-tissue and Multi-Organ)

Hepatocyte Liver Transplantation

These human liver cells are to be transplanted once into patients with liver disease and thus help regenerate their defective organ. But before the healing hepatocytes can be transplanted, they first have to be obtained. And that is not so easy. Because liver cells are very sensitive, it is tricky and expensive to isolate them from donor organs, This is done by perfusing the liver with a special buffer solution, with enzyme solutions, which means the solution runs through the organ, through the vessels, and thereby separates the tissue into a cell suspension. This is cryopreserved, that is, frozen, with a special freezing program, so that these cells can be thawed again at some point and viable liver cells are obtained again after thawing. After numerous experiments with pig livers, the process now also works with human hepatocytes. A cluster of cells can be seen under the microscope: Human liver cells showing all typical functions four days after thawing potential raw material for liver cell therapy. The Hanover researchers want to start the first clinical tests as early as the next six months. But even if they are successful, one drawback will remain: Because the isolated liver cells hardly reproduce in the laboratory, only as much tissue can be transplanted as was previously cut out of a donor organ. Primary adult hepatocytes are not appreciably proliferative in vitro, despite long intensive research. In contrast, stem cells can be derived from regenerable cell resources like bone marrow. Adult stem cells from bone marrow can turn into liver cells after transplantation, this was recently demonstrated in the USA. The decisive advantage here is that because stem cells can be easily multiplied in the laboratory, physicians could have as much healthy liver tissue available as they want in the not too distant future. Paradise conditions for surgeons. But until this dream comes true, researchers must first understand what actually happens when a stem cell transforms into a liver cell. The mechanisms of this cell differentiation are still largely in the dark. And until they are deciphered, clinical application remains risky. So for the time being, donor organs remain the only source of liver cells.

Mesenchymal Stem Cell Transplantation

Mesenchymal stem cells (MSCs) are multipotent progenitor cells of various cell types derived from mesenchyme. They can differentiate into osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells) and adipocytes (fat cells), among others. In addition, differentiation into neurons, astrocytes, and oligodendrocytes (cells of the nervous system) has also been observed. Mesenchymal stem cells have a high proliferation and differentiation potential. Adult mesenchymal stem cells contribute to the maintenance and regeneration of supporting and connective tissues, such as bone, cartilage, muscle, ligaments, tendons, and adipose tissue. They also support the growth and development of blood progenitor cells in the bone marrow (hematopoiesis). MSCs can be isolated from almost all tissues (bone marrow, cartilage, adipose tissue, muscle, liver tissue, blood, amniotic fluid). Due to the very high MSC content as well as the good accessibility, MSC are often isolated from adipose tissue (e.g. from liposuction aspirates). Because of the heterogeneity of purifications, the term mesenchymal stem/stromal cells (rather than mesenchymal stem cells) is now preferred by the scientific community. MSCs can be cultured and differentiated into various cells and tissues in vitro. Specific functional differentiation can be actively controlled by activation or suppression of genes, resulting in the formation of bone marrow stroma supporting hematopoiesis or differentiating into osteogenic, chondrogenic or adipogenic cells. In addition, differentiated MSCs are capable of transforming into another tissue and adapting to novel environmental conditions.

Signaling agents for these regeneration and growth processes are cell-cell contacts and the secretion of growth factors and cytokines. They are mostly obtained from bone marrow (aspiration from iliac crest, also tibia or femur). In stem cell transplantation, the hematopoietic components from bone marrow (or peripheral blood) are transferred to the tumor patient previously treated by total body irradiation or high-dose chemotherapy; MSCs play an essential role in the reconstruction of the destroyed bone marrow.

Hepatic Progenitor Cell Transplantation

Hepatic stellate cells (HSCs) are best known for their involvement in fibrosis development. In contrast, their function in the normal liver has remained largely unclear. Our studies now show that HSCs can be regarded as mesenchymal stem cells (MSCs) and can fulfill their functions. Thus, rat HSC express typical markers of MSC such as nestin, NG2 (neural-glial antigen 2), CD105 (endoglin) as well as CD146 and are able to differentiate into adipocytes as well as osteocytes in vitro.

Furthermore, isolated HSCs support the maintenance as well as differentiation of hematopoietic stem cells in a co-culture system and can thus replace MSC of the bone marrow.

Transplanted eGFP+ HSC, previously purified by FACS (fluorescence-activated cell sorting) based on their retinoid fluorescence, colonize the bone marrow of recipient animals in accordance with their characterization as MSC. After damage to the liver by partial hepatectomy and concomitant application of 2- acetylaminofluorene, FACS-sorted HSC reach the injured liver of the recipient animal and form approximately 11% (± 2%) of the liver tissue after 7 to 14 days. The transplanted HSCs give rise to hepatocytes and cholangiocytes in addition to mesenchymal tissue.

During their differentiation into hepatocytes in vitro, HSCs, like bone marrow- and umbilical cord-derived MSC, transiently undergo the expression profile of hepatic progenitor cells (oval cells), which typically express keratin 19, EPCAM (epithelial cell adhesion molecule), and α-fetoprotein. Finally, in the course of their hepatic differentiation, HSCs and other MSCs exhibit the synthesis of a broad spectrum of bile salts as well as the transporters BSEP (bile salt export pump) and NTCP (sodium taurocholate cotransporting polypeptide) in addition to the release of albumin. These findings indicate for the first time that oval cells arise from stellate cells or other MSCs in the body and thus HSCs may fulfill an important role in liver regeneration.

Multi-Organ)

Acute (ALV), chronic (CLV), and acute-on-chronic liver failure (ACLV) occur frequently in critical care and are associated with high mortality. Since the accumulation and lack of elimination of endogenous and exogenous toxins is one of the main problems in any form of liver failure, the focus today is on the use of detoxification systems that primarily support the detoxification function of the liver and thus indirectly its regeneration. This chapter introduces the MARS ("molecular adsorbents recirculatory system") and Prometheus procedures. However, the standard use of extracorporeal liver support systems cannot be recommended in acute or acute-on- chronic liver failure or alcohol toxic hepatitis. In special situations, however, their use may be helpful, either as a bridge to possible transplantation or until recovery of liver function.

Discussion

Organoids are three-dimensional (3D), multicellular microtissues derived from stem cells and engineered to mimic the complex structure and functionality of a human organ, such as the lung, liver, or brain [1-35]. Organoids are multicellular and exhibit a high degree of self-assembly. They further reproduce complex in vivo cell responses and cell interactions compared to traditional 2D cell cultures. There are three unique definitions that constitute an organoid. It is a 3D biological microtissue that contains different cell types [36-39]. It reflects the complexity, organization and structure of a tissue. It shows similarity to at least one functional aspect of a tissue. Organoids are becoming increasingly important in the fields of cancer research, neurobiology, stem cell research, and drug discovery because they enable improved modeling of human tissues. Because they are generated from stem cells, organoids can be differentiated into a wide range of tissue types, including liver, lung, brain, kidney, stomach and intestinal tissues. Because these 3D microtissues mimic in vivo organs, they can provide researchers with greater insights into the mechanisms of human development and disease. For example, researchers can use organoids from genetically modified cells to determine how certain gene mutations relate to specific genetic diseases. Organoids can also enable the study of infectious diseases and host-pathogen interactions. Last but not least, the ability to use patient-derived organoids for drug screening and toxicity assessments will allow researchers to make further advances in personalized medicine. Due to the increasing complexity of organdies and other 3D cell systems, even more sophisticated techniques for 3D imaging and analysis are required to accurately and efficiently describe these biological structures. Today, automated confocal imaging systems and 3D image analysis software are widely used to help researchers optimize their workflow and achieve optimal results [30,36,39-42]. Self-organization makes life easier for organoid researchers, but it has its limits in vitro. Despite regular divisions, most organoids develop a necrotic core and stop developing [27,36,41,43]. Photoreceptors on the organoid surface, for example, continue to mature after a year, but the internal structure deteriorates over time. This is because diffusion alone cannot provide the metabolic requirements for nutrient and oxygen supply and waste disposal at the center of an organoid. This limits organoids to diameters of three to four millimeters. A number of ideas now exist to delay necrosis. For example, the organoid diameter can be limited - for example, by culturing at an air-liquid interface, with vibratomes, or with artificial chips that physically allow growth in only two spatial directions. None of this, however, solves the actual perfusion problem. That is why vascular networks are considered the holy grail of organoid research [32]. Liebau explains the methodological challenge as even with the addition of endothelial stem cells, organoids are not automatically infused with blood vessels. Growth and organoid growth are different. In vitro self-organization fails here. In embryogenesis, for example, the retina is not directly vascularized either, and blood vessels only grow in later. Replicating this interaction of two entities in vitro while outsmarting biology is difficult. One approach to solving the problem is offered by certain 3D bio-printers, known as inverse extrusion printers. They print networks of 0.4- to 1-millimeter-wide gelatin channels into densely packed clusters of cells, which are then liquified, lined with endothelial cells and flooded with oxygenated medium. From human iPS cells, they grew vascular organoids whose endothelial cells and pericytes formed a capillary network. After transplantation into mice, the capillaries even interconnected with the murine bloodstream and formed perfused arteries, arterioles and venules [1-105]. Three-dimensional tissue models are more produced in the Petri dish to develop blood-liver organoids that can be used to observe disease processes at the cellular and molecular levels [32,41,43,44]. Organoids, often referred to as miniature organs, are cell culture models that represent a tissue in three dimensions in the Petri dish. Researchers usually grow them from stem cells that are not yet or barely differentiated. They can develop into any cell type, such as heart or kidney cells, muscle cells or neurons. In the laboratory, they become liver cells. As in real organ tissue, these are permeated by various blood and immune cells. Liver cells do not develop in a vacuum, but together with white blood cells and other cells of the body's own defense system. And like real liver cells, the liver cells created by his group produce blood - visible as red spots between the rice-grain-sized clusters of cells. Organoids are a kind of window into a previously inaccessible organ, the fetal liver. Tickling liver cells awake with genetic scissors is possible. In organoids, this genetic manipulation works much better than in cells derived

from animal models [42,45]. For example, liver cells that slip into an identity crisis in the course of chronic liver disease and no longer behave like liver cells can be genetically reactivated in the Petri dish. With the help of the gene scissors CRISPR, it is possible to intervene in a liver cell so that it once again does what a liver cell must do. In this way, the miniature organs help reduce the number of animal searches. New gene therapies can first be tested on organoids.

Animal experiments are then only necessary to confirm the experiments in the living organism. Another advantage, he said, is that organoids are available almost indefinitely because their cell source never dries up. In conclusion, organoid research is a very interesting field for the future to treat and cure different pediatric diseases like homocysteinuria or other rare diseases [1-105].

Organoid research will be performed worldwide and it is a challenge for the future to implant organoids into human being and to restore, especially in homocysteinuria, the function of the liver.

Declaration

Acknowledgments

None to declare.

Financial Disclosure

There is no financial disclosure, nor any funding.t

Conflict of Interest

There is no conflict of interest.

Informed Consent

Not applicable.

Author Contributions

SB performed research, data collection and references; EL and GV read the manuscript and gave important ideas; EM checked grammar and style of the manuscript. LB checked the format and the grammar.

Data Availability

Any inquiries regarding supporting data availability of this review should be directed to corresponding author.

References

1. Kruger WD. Cystathionine β-synthase deficiency: Of mice and men. Mol Genet Metab. 2017;121(3):199-205.
2. Elsaid MF, Bener A, Lindner M, et al. Are heterocygotes for classical homocystinuria at risk of vitamin B12 and folic acid deficiency? Mol Genet Metab. 2007;92(1-2):100-3.
3. Majtan T, Pey AL, Ereño-Orbea J, Martínez-Cruz LA, Kraus JP. Targeting Cystathionine Beta-Synthase Misfolding in Homocystinuria by Small Ligands: State of the Art and Future Directions. Curr Drug Targets. 2016;17(13):1455-70.
4. CBS mutations are good predictors for B6-responsiveness: A study based on the analysis of 35 Brazilian Classical Homocystinuria patients. Mol Genet Genomic Med. 2018;6(5):861.
5. Lu YH, Cheng LM, Huang YH, et al. Heterozygous carriers of classical homocystinuria tend to have higher fasting serum homocysteine concentrations than non- carriers in the presence of folate deficiency. Clin Nutr. 2015;34(6):1155-8.
6. Poloni S, Sperb-Ludwig F, Borsatto T, et al. CBS mutations are good predictors for B6-responsiveness: A study based on the analysis of 35 Brazilian Classical Homocystinuria patients. Mol Genet Genomic Med. 2018;6(2):160-170.
7. Majtan T, Jones W Jr, Krijt J, et al. Enzyme Replacement Therapy Ameliorates Multiple Symptoms of Murine Homocystinuria. Mol Ther. 2018;26(3):834-844.
8. Park I, Bublil EM, Glavin F, Majtan T. Interplay of EnzymeTherapy and Dietary Management of Murine Homocystinuria. Nutrients. 2020;12(9):2895.
9. Maclean KN, Jiang H, Aivazidis S, et al. Taurine treatment prevents derangement of the hepatic γ-glutamyl cycle and methylglyoxal metabolism in a mouse model of classical homocystinuria: regulatory crosstalk between thiol and sulfinic acid metabolism. FASEB J. 2018;32(3):1265-1280.
10. Levy HL. Early Development of Newborn Screening for HCU and Current Challenges. Int J Neonatal Screen. 2021;7(4):67.
11. Hart C, McNulty J, Cotter M, Al Jasmi F, Crushell E, Monavari AA. The challenges of pregnancy management in pyridoxine nonresponsive homocystinuria: The Irish experience. JIMD Rep. 2021;61(1):34-41.
12. Sun S, Weile J, Verby M, Wu Y, et al. A proactive genotype-to-patient-phenotype map for cystathionine beta- synthase. Genome Med. 2020;12(1):13.
13. Lawson-Yuen A, Levy HL. The use of betaine in the treatment of elevated homocysteine. Mol Genet Metab. 2006;88(3):201-7.
14. Kerkvliet SP, Rheault MN, Berry SA. Liver transplant as a curative treatment in a pediatric patient with classic homocystinuria: A case report. Am J Med Genet A. 2021;185(4):1247-1250.
15. Oliveira Santos M, Geraldes R, Conceição I. Peripheral nerve involvement in classic homocystinuria: an unusual association. BMJ Case Rep. 2016;2016:bcr2016216255.
16. Sellos-Moura M, Glavin F, Lapidus D, Evans KA, Palmer L, Irwin DE. Estimated prevalence of moderate to severely elevated total homocysteine levels in the United States: A missed opportunity for diagnosis of homocystinuria? Mol Genet Metab. 2020;130(1):36-40.
17. Valayannopoulos V, Schiff M, Guffon N, et al. Betaine anhydrous in homocystinuria: results from the RoCH registry. Orphanet J Rare Dis. 2019;14(1):66.
18. Betaine anhydrous: new drug. Homocystinuria: continued evaluation needed. Prescrire Int. 2009;18(102):162.
19. Majtan T, Pey AL, Gimenez-Mascarell P, et al. Potential Pharmacological Chaperones for Cystathionine Beta-Synthase-Deficient Homocystinuria. Handb Exp Pharmacol. 2018;245:345-383.
20. Majtan T, Pey AL, Ereño-Orbea J, Martínez-Cruz LA, Kraus JP. Targeting Cystathionine Beta-Synthase Misfolding in Homocystinuria by Small Ligands: State of the Art and Future Directions. Curr Drug Targets. 2016;17(13):1455-70.
21. Tan CRC, Abdul-Majeed S, Cael B, Barta SK. Clinical Pharmacokinetics and Pharmacodynamics of Bortezomib. Clin Pharmacokinet. 2019;58(2):157-168.
22. Fricker LD. Proteasome Inhibitor Drugs. Annu Rev Pharmacol Toxicol. 2020;60:457-476.
23. Bublil EM, Martin T. Classical homocystinuria: From cystathionine beta-synthase deficiency to novel enzyme therapies. Biochimie. 2020;173:48-56.
24. Park I, Bublil EM, Glavin F, Majtan T. Interplay of Enzyme Therapy and Dietary Management of Murine Homocystinuria. Nutrients. 2020;12(9):2895..
25. Park I, Hůlková H, Krijt J, Kožich V, Bublil EM, Majtan T. Long-term uninterrupted enzyme replacement therapy prevents liver disease in murine model of severe homocystinuria. Hum Mutat. 2020 Sep;41(9):1662-1670.
26. Pegtibatinase as an Enzyme Therapy for Patients WithHomocystinuria Caused by Cystathionine Beta-Synthase

Deficiency (COMPOSE); Interventional clinical trial in 32 participants: A Double Blind, Randomized, Placebo-controlled, Phase 1/2 Study to Assess the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Effects on Clinical Outcomes of Pegtibatinase (TVT-058), Administered Subcutaneously in Patients With Cystathionine Beta-Synthase Deficient Homocystinuria (COMPOSE), ClinicalTrials.gov Identifier: NCT03406611

27. A Multiple Ascending Dose Study of ACN00177 (Pegtarviliase) in Subjects With CBS Deficiency; A Phase 1/2 Multiple Ascending-Dose Study in Subjects With Homocystinuria Due to Cystathionine β- Synthase (CBS) Deficiency to Investigate the Safety, Pharmacokinetics, and Pharmacodynamics of ACN00177. ClinicalTrials.gov Identifier: NCT05154890
28. Levy HL. Early Development of Newborn Screening for HCU and Current Challenges. Int J Neonatal Screen. 2021;7(4):67.
29. Lee IS, Takebe T. Narrative engineering of the liver. Curr Opin Genet Dev. 2022;75:101925.
30. Wei Y, Wang YG, Jia Y, et al. Liver homeostasis is maintained by midlobular zone 2 hepatocytes. Science. 2021;371(6532):eabb1625..
31. Jia Y, Li L, Lin YH, et al. In vivo CRISPR screening identifies BAZ2 chromatin remodelers as druggable regulators of mammalian liver regeneration. Cell Stem Cell. 2022 Mar 3;29(3):372-385.e8.
32. Chan C, Berthiaume F, Nath BD, Tilles AW, Toner M, Yarmush ML. Hepatic tissue engineering for adjunct and temporary liver support: critical technologies. Liver Transpl. 2004;10(11):1331-42.
33. Smets F, Dobbelaere D, McKiernan P, et al. Phase I/II Trial of Liver-derived MesenchymalStem Cells in Pediatric Liver-based Metabolic Disorders: A Prospective, Open Label, Multicenter, Partially Randomized, Safety Study of One Cycle of Heterologous Human Adult Liver-derived Progenitor Cells (HepaStem) in Urea Cycle Disorders and Crigler- Najjar Syndrome Patients. Transplantation. 2019; 103: 1903–15.
34. Stevens KR, Scull MA, Ramanan V, et al. In situ expansion of engineered human liver tissue in a mouse model of chronic liver disease. Sci Transl Med. 2017; 9.
35. Takebe T, Enomura M, Yoshizawa E, et al. Vascularized and Complex Organ Buds from Diverse Tissues via Mesenchymal Cell- Driven Condensation. Cell Stem Cell. 2015;16:556–65.
36. Meran L, Massie I, Campinoti S, et al. Engineering transplantable jejunal mucosal grafts using patient-derived organoids from children with intestinal failure. Nat Med. 2020;26(10):1593-1601.
37. Koike H, Iwasawa K, Ouchi R, et al. Engineering human hepato-biliary-pancreatic organoids from pluripotent stem cells. Nat Protoc. 2021;16(2):919-936.
38. Takebe T, Zhang RR, Koike H, et al. Generation of a vascularized and functional human liver from an iPSC-derived organ bud transplant. Nat Protoc. 2014;9: 396–409.
39. Mori A, Murata S, Tashiro N, et al. Establishment of Human Leukocyte Antigen-Mismatched Immune Responses after Transplantation of Human Liver Bud in Humanized Mouse Models. Cells. 2021; 10: 476.
40. Thompson J, Jones N, Al-Khafaji A, et al. Extracorporeal cellular therapy (ELAD) in severe alcoholic hepatitis: A multinational, prospective, controlled, randomized trial. Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society. 2018;24:380–93.
41. Prior N, Hindley CJ, Rost F, et al. Lgr5(+) stem and progenitor cells reside at the apex of a heterogeneous embryonic hepatoblast pool. Development. 2019; 146.
42. Takebe T, Wells JM, Helmrath MA, Zorn AM. Organoid Center Strategies for Accelerating Clinical Translation. Cell Stem Cell. 2018;22:806–9.
43. Saborowski A, Wolff K, Spielberg S, et al. Murine Liver Organoids as a Genetically Flexible System to Study Liver Cancer In Vivo and In Vitro. Hepatology communications. 2019; 3: 423–36.
44. Ye S, Boeter JWB, Mihajlovic M, et al. A Chemically Defined Hydrogel for Human Liver Organoid Culture. Advanced Functional Materials. 2020;30:2000893.
45. Tsuchida T, Murata S, Hasegawa S, et al. Investigation ofClinical Safety of Human iPS Cell-Derived Liver Organoid Transplantation to Infantile Patients in Porcine Model. Cell transplantation. 2020;29: 963689720964384-.
46. Witzigmann D, Kulkarni JA, Leung J, Chen S, Cullis PR, van der Meel R. Lipid nanoparticle technology for therapeutic gene regulation in the liver. Adv Drug Deliv Rev. 2020;159:344-363.
47. Jang KJ, Otieno MA, Ronxhi J, et al. Reproducing human and cross-species drug toxicities using a Liver-Chip. Sci Transl Med. 2019;11(517):eaax5516.
48. Sun L, Wang Y, Cen J, et al. Modelling liver cancer initiation with organoids derived from directly reprogrammed human hepatocytes.Nat Cell Biol. 2019;21(8):1015-1026.
49. Tam PKH, Wong KKY, Atala A, et al. Regenerative medicine: postnatal approaches. Lancet Child Adolesc Health. 2022;6(9):654-666.
50. Bhatia SN, Underhill GH, Zaret KS, Fox IJ. Cell and tissue engineering for liver disease. Sci Transl Med. 2014;6(245):245sr2.
51. Vacanti JP, Kulig KM. Liver cell therapy and tissue engineering for transplantation. Semin Pediatr Surg. 2014;23(3):150-5.
52. Shi Q, Yang X, Greenhaw JJ, Salminen AT, Russotti GM, Salminen WF. Drug-Induced Liver Injury in Children: Clinical Observations, Animal Models, and Regulatory Status. Int J Toxicol. 2017;36(5):365-379.
53. Xu M, Tan J, Dong W, et al. The E3 ubiquitin-protein ligase Trim31 alleviates non-alcoholic fatty liver disease by targeting Rhbdf2 in mouse hepatocytes. Nat Commun. 2022;13(1):1052.
54. Villiger L, Grisch-Chan HM, Lindsay H, et al. Treatment of a metabolic liver disease by in vivo genome base editing in adult mice. Nat Med. 2018;24(10):1519-1525.
55. Kaltenbacher T, Löprich J, Maresch R, et al. CRISPR somatic genome engineering and cancer modeling in the mouse pancreas and liver. Nat Protoc. 2022;17(4):1142-1188.
56. Shinozawa T, Yoshikawa HY, Takebe T. Reverse engineering liver buds through self-driven condensation and organization towards medical application. Dev Biol. 2016;420(2):221-229.
57. Mazza G, Al-Akkad W, Telese A, et al. Rapid production of human liver scaffolds for functional tissue engineering by high shear stress oscillation-decellularization. Sci Rep. 2017;7(1):5534.
58. Nevens F, Gustot T, Laterre PF, et al. A phase II study of human allogeneic liver-derived progenitor cell therapy for acute-on-chronic liver failure and acute decompensation. JHEP Rep. 2021;3(4):100291.
59. Lo RC-L, Ng IO-L. Hepatic progenitor cells: their role and functional significance in the new classification of primary liver cancers. Liver Cancer. 2013;2:84–92.
60. Stevens KR, Schwartz RE, Ng S, Shan J, Bhatia SN. Chapter 46 - Hepatic Tissue Engineering. In: Lanza R, Langer R, Vacanti J, eds. Principles of Tissue Engineering (Fourth Edition). Academic Press, Boston, 2014: 951–86.
61. Huang YK, Tan DM, Xie YT, et al. Randomized controlled study of plasma exchange combined with molecular adsorbent re-circulating system for the treatment of liver failure complicated with hepatic encephalopathy. Hepatogastroenterology. 2012;59:1323–6.
62. Kribben A, Gerken G, Haag S, et al. Effects of fractionated plasma separation and adsorptionon survival in patients with acute-on-chronic liver failure. Gastroenterology. 2012;142:782–9.e3.
63. Lee S, Lee J-H, Lee D-H, et al. Phase 1/2a Trial of a Bioartificial Liver Support System (LifeLiver) for Acute Liver Failure Patients. Transplantation. 2018; 102.
64. Wu G, Wu D, Lo J, et al. A bioartificial liver support system

integrated with a DLM/GelMA based bioengineered whole liver for prevention of hepatic encephalopathy via enhanced ammonia reduction. Biomaterials Science. 2020;8:2814–24.

65. Hou Y-T, Hsu C-C. Development of a 3D porous chitosan/gelatin liver scaffold for a bioartificial liver device. Journal of Bioscience and Bioengineering. 2020;129:741–8.
66. Pyrsopoulos NT, Hassanein T, Subramanian R, et al. THU-272-A study investigating the effect of extracorporeal cellular therapy with C3A cells on the survival of alcoholic hepatitis designed along the guidelines of the NIAAA. Journal of Hepatology. 2019;70:e282.
67. Pan X-P, Li L-J. Advances in cell sources of hepatocytes for bioartificial liver. Hepatobiliary & Pancreatic Diseases International. 2012;11:594–605.
68. Takeishi K, Collin de l'Hortet A, Wang Y, et al. Assembly and Function of a Bioengineered Human Liver for Transplantation Generated Solely from Induced Pluripotent Stem Cells. Cell reports. 2020; 31:107711.
69. Chamuleau RA, Poyck PP, Van De Kerkhove M-P. Bioartificial Liver: Its Pros and Cons. Therapeutic Apheresis and Dialysis. 2006;10:168–74.
70. Marsee A, Roos FJM, Verstegen MMA, et al. Building consensus on definition and nomenclature of hepatic, pancreatic, and biliary organoids. Cell Stem Cell. 2021;28:816–32.
71. Mitaka T, Sato F, Mizuguchi T, Yokono T, Mochizuki Y. Reconstruction of hepatic organoid byrat small hepatocytes and hepatic nonparenchymal cells. Hepatology. 1999;29:111–25.
72. Mitaka T. Reconstruction of hepatic organoid by hepatic stem cells. Journal of Hepato-Biliary Pancreatic Surgery. 2002;9:697–703.
73. Schneeberger K, Sánchez-Romero N, Ye S, et al. Large-Scale Production of LGR5-Positive Bipotential Human Liver Stem Cells. Hepatology. 2020; 72: 257–70.
74. Hu H, Gehart H, Artegiani B, et al. Long-Term Expansion of Functional Mouse and Human Hepatocytes as 3D Organoids. Cell. 2018;175:1591–606.e19.
75. Yamamoto J, Udono M, Miura S, Sekiya S, Suzuki A. Cell Aggregation Culture Induces Functional Differentiation of Induced Hepatocyte-like Cells through Activation of Hippo Signaling. Cell Rep. 2018;25:183–98.
76. Camp JG, Sekine K, Gerber T, et al. Multilineage communication regulates human liver bud development from pluripotency. Nature. 2017;546:533–8.
77. Akbari S, Sevinç GG, Ersoy N, et al. Robust, Long-Term Culture of Endoderm-Derived Hepatic Organoids for Disease Modeling. Stem Cell Reports. 2019;13:627–41.
78. Koike H, Iwasawa K, Ouchi R, et al. Modelling human hepato-biliary-pancreatic organogenesis from the foregut-midgut boundary. Nature. 2019;574:112–6.
79. Tapia N, Schöler HR. Molecular Obstacles to Clinical Translation of iPSCs. Cell Stem Cell. 2016;19: 298–309.
80. Sun L, Hui L. Progress in human liver organoids. J Mol Cell Biol. 2020;12(8):607-617.
81. Zhou VX, Lolas M, Chang TT. Direct orthotopic implantation of hepatic organoids. J Surg Res. 2017; 211:251–60.
82. Wang S, Wang X, Tan Z, et al. Human ESC-derived expandable hepatic organoids enable therapeutic liver repopulation and pathophysiological modeling of alcoholic liver injury. Cell Res. 2019;29:1009–26.
83. Pettinato G, Lehoux S, Ramanathan R, et al. Generation of fully functional hepatocyte-like organoids from human induced pluripotent stem cells mixed with Endothelial Cells. Sci Rep. 2019;9:8920.
84. Kruitwagen HS, Oosterhoff LA, van Wolferen ME, et al. Long-Term Survival of Transplanted Autologous Canine Liver Organoids in a COMMD1-Deficient Dog Model of Metabolic Liver Disease. Cells. 2020;9(2):410.
85. Tsuchida T, Murata S, Matsuki K, et al. The Regenerative Effect of Portal Vein Injection of Liver Organoids by Retrorsine/Partial Hepatectomy in Rats. Int J Mol Sci. 2019;21:178.
86. Sampaziotis F, Justin AW, Tysoe OC, et al. Reconstruction of the mouse extrahepatic biliary tree using primary human extrahepatic cholangiocyte organoids. Nat Med. 2017;23:954–63.
87. Sampaziotis F, Muraro D, Tysoe OC, et al. Cholangiocyte organoids can repair bile ducts after transplantation in the human liver. Science. 2021;371:839–46.
88. Takebe T, Sekine K, Kimura M, et al. Massive and Reproducible Production of Liver Buds Entirely from Human Pluripotent Stem Cells. Cell Reports. 2017;21:2661–70.
89. Saito R, Ishii Y, Ito R, et al. Transplantation of liver organoids in the omentum and kidney. Artif Organs. 2011;35:80–3.
90. Nie YZ, Zheng YW, Ogawa M, Miyagi E, Taniguchi H. Human liver organoids generated with single donor-derived multiple cells rescue mice from acute liver failure. Stem Cell Res Ther. 2018;9:5.
91. Harrison SP, Siller R, Tanaka Y, et al. Scalable production of tissue-like vascularised liver organoids from human PSCs. bioRxiv. 2020: 2020.12.02.406835.
92. Nussler A, Konig S, Ott M, et al. Present status and perspectives of cell-based therapies for liver diseases. J Hepatol. 2006; 45: 144–59.
93. Sekine K, Ogawa S, Tsuzuki S, et al. Generation of human induced pluripotent stem cell-derived liver buds with chemically defined and animal origin-free media. Scientific Reports. 2020;10:17937.
94. Cristinziano G, Porru M, Lamberti D, et al. FGFR2 fusion proteins drive oncogenic transformation of mouse liver organoids towards cholangiocarcinoma. J Hepatol. 2021;75(2):351-362.
95. Cao W, Liu J, Wang L, et al. Modeling liver cancer and therapy responsiveness using organoids derived from primary mouse liver tumors. Carcinogenesis. 2018; 40:145–54.
96. Pettinato G, Lehoux S, Ramanathan R, et al. Generation of fully functional hepatocyte-like organoids from human induced pluripotent stem cells mixed with Endothelial Cells. Sci Rep. 2019;9(1):8920.
97. Kruitwagen HS, Oosterhoff LA, van Wolferen ME, et al. Long-Term Survival of Transplanted Autologous Canine Liver Organoids in a COMMD1-Deficient Dog Model of Metabolic Liver Disease. Cells. 2020;9:410.
98. Zhang R-R, Koido M, Tadokoro T, et al. Human iPSC-Derived Posterior Gut Progenitors Are Expandable and Capable of Forming Gut and Liver Organoids. Stem Cell Reports. 2018;10:780–93.
99. Chen C, Soto-Gutierrez A, Baptista PM, Spee B. Biotechnology Challenges to In Vitro Maturation of Hepatic Stem Cells. Gastroenterology. 2018;154:1258–72.
100. Liu J, Li P, Wang L, et al. Cancer-Associated Fibroblasts Provide a Stromal Niche for Liver Cancer Organoids That Confers Trophic Effects and Therapy Resistance. Cell Mol Gastroenterol Hepatol. 2021;11:407–31.
101. Kim SK, Kim YH, Park S, Cho SW. Organoid engineering with microfluidics and biomaterials for liver, lung disease, and cancer modeling. Acta Biomater. 2021;132:37-51.
102. Yap KK, Gerrand Y-W, Dingle AM, Yeoh GC, Morrison WA, Mitchell GM. Liver sinusoidal endothelial cells promote the differentiation and survival of mouse vascularised hepatobiliary organoids. Biomaterials. 2020;251:120091.
103. Yanagi Y, Nakayama K, Taguchi T, et al. In vivo and ex vivo methods of growing a liver bud through tissue connection. Sci Rep. 2017;7(1):14085.
104. Grebenyuk S, Ranga A. Engineering Organoid Vascularization. Front Bioeng Biotechnol. 2019;7:39.
105. Eiji K, Shin E. Liver bud transplantation in rats. Magyar Sebészet (Hungarian Journal of Surgery) MaSeb. 2018; 71: 163–9.