Present and Future Perspectives of Using Human-Induced Pluripotent Stem Cells and Organoid Against Liver Failure

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Abstract
Organ failure manifests severe symptoms affecting the whole body that may cause death. However, the number of organ donors is not enough for patients requiring transplantation worldwide. Illegal transplantation is also sometimes conducted. To help address this concern, primary hepatocytes are clinically transplanted in the liver. However, donor shortage and host rejection via instant blood-mediated inflammatory reactions are worrisome. Induced pluripotent stem cell-derived hepatocyte-like cells have been developed as an alternative treatment. Recently, organoid technology has been developed to investigate the pathology and mechanism of organoids in cultures. Organoids can be transplanted with vascularization and connected to host blood vessels, and functionally mature better in vivo than in vitro. Hepatic organoids improve pathology in liver disease models. In this review, we introduce induced pluripotent stem cell- and organoid-based therapies against liver diseases considering present and future perspectives.

Keywords
Stem cell therapy, liver regeneration

Introduction
Liver tissues can regenerate on being injured. Acute liver failure has different etiologies, including drug overdose, viral infection, ischemia, etc., and has a high mortality rate¹. In contrast, chronic liver injury results from viral infection, alcoholism, nonalcoholic steatohepatitis, autoimmune disorders, metabolic diseases, and promotes liver fibrosis². Although the liver has considerable regenerative potential, it cannot regenerate when there is chronic fibrosis or cirrhosis. Early fibrosis can be reversible, whereas cirrhosis is irreversible. In 2012, cirrhosis was the 14th leading cause of death worldwide³. Moreover, it can cause hepatocellular carcinoma, which is the most common metastatic liver cancer. Organ transplantation is the only treatment option for both acute liver failure and end-stage liver disease. Transplantation increases the chance of survival in patients with acute-on-chronic liver failure (ALF) grades 2 and 3⁴. Another report showed that the hospital survival rate of patients with ALF who underwent transplantation increased from 16.7% to 62.2%⁵.

Progress of Organ Transplantation
Organ transplantation is the only treatment option for the heart, kidney, and liver at the terminal state of organ failure. Although the number of patients on the waiting list for organ transplantation continues to increase, the supply of transplantable organs cannot sufficiently meet the demand. Illegal transplantation may be performed in 10% of all patients to satisfy a large demand⁶. One reason is that many deceased organs are not transplantable because the donors are at high risk. For example, the donor was declared dead based on cardiovascular criteria, as opposed to brainstem death donors, or the donor was elderly with multiple comorbidities (extended criteria donors)⁷. In addition, over about 30 years, there has been no advancement in the methods of organ preservation. Organs are usually stored in an icebox, called...
static cold storage (SCS), to slow down the metabolism. However, when the organ is transplanted, ischemia-reperfusion generates reactive oxygen species, which damage the transplanted organ. To resolve this, machine perfusion was developed, and the first randomized controlled trial was performed. This novel technique increases the duration of organ storage and maintains the physiological function of the organ. Compared with SCS, machine perfusion increases the storage life of transplantable organs. Moreover, an inadequate number of donors is predicted even by using machine perfusion.

### Hepatocyte Transplantation

An alternative approach to organ transplantation is transplantation of hepatocytes. Hepatocytes are transplanted because they can repair and replace the host liver. They are obtained via patients autopsy then cryoprotected. Transplantation of hepatocytes is optimal for liver tissue; however, donor shortages and instant blood-mediated inflammatory reaction (IBMIR) are difficulties faced by medical professionals during hepatocyte transplantation. IBMIR recognizes transplanted hepatocytes and rejects them through the activation of both complement and coagulation pathways. Moreover, hepatocytes have low viability and little proliferation capability in cultures despite recent improvement in culture methods. Cryopreservation tends to be deleterious in viability, attachment, and engraftment. Despite their functionality, hepatocytes show low engraftment and are difficult to preserve, which is a matter of concern.

### Somatic Stem Cell Transplantation

As an alternative cell transplantation, several types of stem cells have been reported as resources to restore liver functions. Bone marrow-derived cells differentiate into hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and endothelial progenitor cells (EPCs). Several studies have proven the feasibility of HSCs, MSCs, and EPCs in restoring hepatic functions in liver injury models. MSCs are beneficial for transplantation. They can be obtained from many tissues including bone marrow, umbilical cord blood, adipose tissue, and placenta, and are easily cultured ex vivo. Moreover, they have immunomodulatory properties. In fact, the safety and short-term efficacy of transplantation of bone marrow-derived MSCs was reported to significantly improve Child–Pugh and model for end-stage liver disease (MELD) scores in 20 patients. In total, 40 registered trials targeting liver cirrhosis or acute liver diseases have used different types of MSCs. However, the results of most of these trials showed only temporary effects and need to be further investigated with large cohorts. Furthermore, MSCs cannot reconstitute the host liver.

### Pluripotent Stem Cell Transplantation

Embryonic stem (ES) cells are pluripotent and can differentiate into hepatocyte-like cells. They demonstrate mature hepatocyte-like properties. ES cell-derived hepatocyte-like cells contribute to liver repair by cell replacement. The generation of induced pluripotent stem (iPS) cells by Takahashi and Yamanaka has resulted in the expansion of iPS cell-based research. Diverse types of cells in the body are differentiated from iPS cells, and each cell shows specific morphology and gene and protein marker expressions and functions.

Research in clinical applications such as cell replacement, disease model or disease-specific iPS cell model (genetic mutation), and drug screening is progressing. Autologous transplantation of iPS cell-derived retinal pigment epithelium was performed for treating neovascular age-related macular degeneration in the eyes, and no serious side effect was reported at 25 months of follow-up in one patient. Moreover, a clinical trial was started for Parkinson’s disease. In 2018, Kikuchi et al. implanted 2.4 million dopamine precursor cells into the brain of a patient with Parkinson’s disease. They reported that the transplanted dopamine precursor cells were functional in the primate brain model of Parkinson’s disease. iPS-based clinical application has progressed to confirm its safety and efficacy.

In a basic research study, transplantation of human iPS cell-derived hepatocyte-like cell sheets was reported. This sheet was made using temperature-responsive culture dishes, which is a scaffoldless technology with clinical applications. Sheet transplantation ameliorates the lethal acute liver injury induced by carbon tetrachloride in mice. Furthermore, recently, iPS cells have been reported to generate liver-specific endothelial (sinusoidal) cells and stellate cells. Moreover, liver parenchymal and non-parenchymal cells can be induced from iPS cells in two-dimensional cultures. The differentiation of ES or iPS cells to hepatocytes in vitro is successful and iPS-hepatocyte like cells show therapeutic effect in vivo. However, two-dimensional hepatocytes cannot be produced in sufficient numbers, and are not sufficient to reconstitute the liver, which is a complex and large tissue with different cell types.

### Functional Three-Dimensional Organoids

The liver is a complex tissue mainly composed of hepatocytes, liver sinusoidal cells, stellate cells, and Kupffer cells and has different functions, such as the production of bile, albumin, cholesterol, and immune factors; glucose storage and release; processing of hemoglobin; and clearance of ammonia and bilirubin. The other organs also have several functions. Therefore, to generate multifunctional tissues, such as liver tissues, three-dimensional (3D) sphere or organoid technology has been developed.

Recent advances show the self-organization of neural cells into multiple layers in the eyes and brain. This
suggests that complex tissues can be reproduced using organoids. However, these organoids are similar to fetal organs but not to adult organs. Functional and mature organoids are preferable for organ replacement. Novel techniques permit expansion of hepatocytes in long-term culture embedded in Matrigel. Some growth factors or TNF-α enable proliferation of hepatocyte organoids in vitro and the repopulation of human hepatocytes after engraftment into a liver injury mouse model.

Another interesting topic of research is organ-on-a-chip technology. This technology enables moderate perfusion and organ–organ interactions in a microfluidic device. Primary human hepatocytes form microtissues with hepatic functions such as albumin secretion and metabolic activity.

To create a complex organ system, researchers have tried to combine organoids with different organs, such as vasculature and nerves. One of the breakthroughs is the generation of vascularized organoids. Takebe et al. created the vascularized and functional human liver bud (LB) from human iPS cells. iPS cell-derived hepatic cells self-organize into 3D iPS-LB by recapitulating interactions during organogenesis with endothelial (human umbilical vein endothelial) and mesenchymal (mesenchymal stem) cells. In 2017, three types of cells, hepatic endoderm, endothelial, and mesenchymal cells, were successfully differentiated from iPS cells and self-organized into LB. Human endothelial cells in iPS-LB become functional blood vessels when connected to the host vessels. Although iPS-LB resembles fetal liver tissue in vivo, LB matures after in vivo transplantation and blood perfusion into LB to escape hypoxia. Moreover, gene expressions in 3D culture are different to those in 2D culture, and gene expressions in the former are similar to those in fetal hepatocytes. A mouse model with lethal liver failure could be treated by transplanting iPS-LB. This demonstrates the generation of a functional human organ-like tissue from iPS cells. Its effects have spread further. This organ bud formation is also driven by mesenchymal cells from other organs such as intestines, lungs, heart, kidneys, and brain, and even cancer cells.

Table 1. Comparison of culture and transplantation properties of each discussed method to produce liver tissue.

|                         | Cell Transplantation | Cell sheet | Organ-on-chip | 3D organoid |
|-------------------------|----------------------|------------|---------------|-------------|
| Generation method       | Obtained from donor; culture and/or differentiation on coating dish | Differentiation on temperature-responsive culture dishes | Cultured in chambers | Cultured in or on Matrigel or matrix-free |
| Cell proliferation and expansion | Almost none | Almost none | Proliferate but limited in size | Proliferate and expand |
| Maturation               | High                | High       | Low maturation | Low but mature in vivo |
| Technical accessibility  | Easy                | Easy       | Hard          | Relatively easy |
| Vascularization          | Absent              | Absent     | Present       | Present     |
| Perfusion                | Absent              | Absent     | Present       | Absent       |
| Transplantation          | Easy                | Easy       | Hard (or impossible) | Relatively easy |

3D: three dimensional.

Figure 1. Overview of the culture process from cell to organ. In two-dimensional cultures, primary hepatocytes, embryonic stem (ES), or induced-pluripotent stem (iPS)-hepatocyte-like cells and iPS-hepatocyte-like cell sheets are developed. In three-dimensional cultures, the functional and vascularized organoid is advanced, and transplantation helps achieve maturation in vivo by blood perfusion.

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investigating the pathology and mechanism of various diseases in each organ. The organ bud is also a potential therapeutic option against some diseases. Moreover, the culture system is continuously advancing, thus progressing from cells to complex organoids (Table 1)44. Specially, organoid technology is progressed by modifying the medium, scaffold, and types of mixed cells. Liver organoid growth is desired in vitro because the liver is a large tissue in the body. Many researchers have partially achieved in vitro growth of hepatocytes since 197645,46,50,54. Advances in organ culture could lead to ex vivo hepatocyte growth, which may be sufficient for the replacement of a host liver. However, there are some hurdles such as low engraftment and high costs to accomplish clinical applications55.

Conclusion and Perspectives

iPS cell-based research and organoid technology have rapidly advanced and aimed at the reconstitution of organs in the past decade (Figure 1). Most recently, several types of organoids have been transplanted to mature or to create a disease model in vivo, even if they are not intended for therapy56–58. Organoid technology is a powerful tool for the establishment of disease models and drug screening. In addition, iPS cell-derived hepatocytes and hepatic organoids are beneficial in the field of regenerative medicine59,60. An organoid is particularly expected to become a functional organ in the host tissue because it is more complex and functional than a single cell population. In the liver, mass production of organoids is essential for treating chronic fibrosis and cirrhosis because most liver tissues cannot regenerate under these conditions, although the recent iPS-LB transplantation method can treat acute liver injury. Perhaps one of the solutions to generating a more functional organoid in vitro is the combination of organoid technologies and perfusion by organs-on-a-chip to reveal the complex pathology and mechanisms60. Some technologies can concordantly accelerate organoid growth and maturation for clinical applications.

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