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Embryonic and Induced Pluripotent Stem Cells as a Model for Liver Disease

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Abstract

Induced pluripotent stem (iPS) cells are human somatic cells that have been reprogrammed to a pluripotent state. Through several elegant technologies, we are now able to generate human iPS cells with disease genotypes that could serve as invaluable tools for human disease modeling. This could lead to an understanding of the root causes of a disease and to the development of effective prophylactic and therapeutic strategies for it. However, we are still far from generating fully functional liver cells from stem cells, including iPS cells, on in vitro culture systems. Tissue-engineering techniques have opened the window to inducing a functional fate for differentiated cells by providing a microenvironment that allows the maintenance of signals similar to those found in the natural microenvironment. Here we review the current technology to establish iPS cells and discuss strategies to generate human liver disease modeling using iPS cell technology in concert with bioengineering approaches.

Keywords

embryonic stem cell; induced pluripotent stem cell; decellularized liver; extracellular matrix and differentiation

I. INTRODUCTION

Since 2006, when Yamanaka's laboratory¹ first reported that four transcription factors, Oct-3/4 (octamer-binding transcription factor-3/4), Sox2 (SRY-related high-mobility-group [HMG]-box protein-2), c-Myc, and Klf4 (Kruppel-like factor-4), can reprogram adult fibroblast cells back to a pluripotent state, interest in induced pluripotent stem (iPS) cells has exploded. The method is striking in that it can convert somatic cells directly into pluripotent cells in a manner that is totally independent of the availability of embryonic cells. Since

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then, iPS cells have become especially attractive for researchers in all areas of regenerative medicine.

Although iPS cells face major hurdles on the road to clinical application, the promise of the production of human cells for research to understand diseases and therapy is enormous. One immediate challenge is that current reprogramming methods involve the expression of putative oncogenes by retroviral or lentiviral vectors, which may themselves cause cancer by integrating into the genome in a way that disrupts endogenous gene expression.^{2,3} To resolve the problem, efforts have been undertaken to produce ideal pluripotent stem cells without the use of viral vectors to deliver the required transcription factors.^{2,3} These methods avoid the potential problem of viral vectors integrating into the recipient cell genome and possibly causing unpredictable genetic dysfunction. The elimination of viral vectors could substantially improve the usefulness and safety of iPS cells as tools in the study of human disease.

iPS cell technology provides opportunities for studying normal development and for drug screening. In addition, researchers have been able to show their potential as a starting point for generating disease models, as iPS cell lines have been produced from patients with neuro-degenerative disorders such as amyotrophic lateral sclerosis⁴ and spinal muscular atrophy.⁵ Furthermore, these cell lines have been successfully differentiated into disease-specific motor neurons, which allow us to follow the disease process or replay the disease in the culture dish using human cells. However, many diseases may not be cell autonomous, and it is still uncertain whether “diseases in a Petri dish” emulate the developmental progression that happens in situ in the human.⁶ Thus, the use of technology available from tissue and organ engineering and/or animal models to regenerate tissues using iPS cells is of great value.

To generate an adequate condition in vitro, defined growth conditions with specific human extracellular matrix components is essential for the maintenance of many kinds of cells.⁷⁻¹⁰ To optimize maturation of differentiated cell lineage from embryonic stem cells (ESCs) or iPS cells, these conditional supports may be required. This review will provide an overview of complementary approaches, iPS cell technology, tissue engineering, and the use of animal models of liver repopulation currently under active investigation to enhance our understanding of liver disease and our efforts to discover successful drugs and cell therapies.

II. THE MANY WAYS TO MAKE iPS CELLS

iPS cells are the product of somatic cell reprogramming to an embryonic-like state. This occurs by the introduction of a defined and limited set of transcription factors and by culturing these cells under ESC conditions.¹ The method was first described by Yamanaka et al. using mouse fibroblasts, in which it was demonstrated that the retroviral-mediated introduction of four transcription factors, Oct3/4, Sox2, c-Myc 1, and Klf4, could induce pluripotency.

Initial generations of mouse and human iPS cells employed retroviral vectors^{1,11} and constitutive lentiviruses,^{12,13} while later generations were produced using inducible lentiviruses.¹⁴⁻¹⁷ These viral systems, however, have been criticized for their permanent integration into the genome. Indeed, the use of integrating viruses for iPS cell induction has represented a major roadblock, because genomic insertion has been shown to alter gene function,¹⁸ and viral transgene reactivation in iPS cell-derived chimeric mice has been implicated in tumorigenesis.¹⁹ Transient overexpression of the four transcription factors should circumvent tumor formation and, possibly, perinatal death, as observed in iPS cell-derived chimeric mice, which are both thought to be caused by ectopic expression of the above four factors.^{20,21}

To reduce these possibilities, small molecules that possibly take the place of reprogramming transgenes have been identified. The use of small molecules and soluble factors is particularly appealing, given their ease of use and lack of permanent genome modification that constrains the use of retroviruses and lentiviruses. Such compounds could also increase the overall efficiency of a transfection, allowing integration-free reprogramming of human cells. Mouse embryonic fibroblasts reprogrammed with the four previously mentioned transcription factors and treated with an inhibitor of methyltransferase (5'-azacytidine) showed a 10-fold increase in reprogramming efficiency.²² More significantly, one of the reprogramming factors could be replaced by a small molecule, enabling the generation of human iPS cells with only two factors. Huangfu et al.²³ exploited the common retroviral delivery method to determine whether small molecules can replace one or more of the reprogramming factors. They found that adding just two factors (Oct4 and Sox2) in combination with a histone deacetylase inhibitor, valproic acid, was sufficient to reprogram human fibroblasts to an embryonic state, suggesting that the rate-limiting step in the process of reprogramming is related to chromatin accessibility. Valproic acid has now been shown to dramatically increase the efficiency of reprogramming in both human and mouse cells¹² irrespective of whether it is induced by just two, three, or four factors. At present, it is generally thought that, eventually, a combination of small molecules will be discovered that can reprogram somatic cells to an embryonic state without the use of transgenes.

The use of cells that express endogenous expression of certain reprogramming factors has also been achieved in order to reduce the number of transgenes delivered into a cell. For example, Kim et al. reported that only two factors, Oct3/4 and Klf4, are required for the generation of iPS cells from neural stem cells, which already have high endogenous expression of Sox2,²⁴ although the success occurs at a lower efficiency than with four factors.²⁵

While some groups have developed methods that eliminate the need for viral transduction or decrease the number of transgenes used,^{26,27} others²⁸ have focused on reprogramming techniques that do not result in genomic integration. These induction methods are considered to be safer and involve the use of adenoviral vectors,²⁶ sendaviral vectors, nonviral plasmids,²⁷ and minicircle vectors.²⁸

Two groups have successfully reprogrammed fibroblasts using a strategy that allows the DNA insertion of all four transcription factors in one transgene, followed by the enzymatic removal of the transgene via cre/transposase-based method of excision after successful reprogramming.^{2,29} This methodology provides a solution to problems associated with retroviral/foreign DNA insertions during iPS-cell generation.

Other approaches to avoid insertional mutagenesis are the use of direct protein induction procedures. Kim et al.²⁵ have recently reported the use of Oct4, Sox2, Klf4, and c-Myc proteins fused with cell-penetrating peptide anchors to successfully reprogram human newborn fibroblasts. However, compared with virus-based techniques, this protocol has a lower reprogramming efficiency of 0.001%.

Thus, several methods have been investigated to induce iPS cells and include different cell types to reprogram, reprogramming a combination of genes, and gene transfer.^{12,13,19,23,30,31} It is now critically important that iPS cells generated by distinct methods be carefully assessed for their variability, stability, and differentiation potential for transplantation studies.

Recent work has demonstrated that there is a strong influence of cell type on reprogrammability, including the efficiency and kinetics of the process, as well as the ease at which reprogramming factors can be delivered to embryonic and tail-tip fibroblasts in the

mouse and dermal fibroblasts in human subjects. These have been the most widely used cell types for reprogramming, largely due to their availability and easy accessibility. In addition, various other cell types have also been reprogrammed, including stomach cells,²⁰ B-lymphocytes,³² pancreatic β -cells,³³ neural stem cells, and hepatocytes²⁶ in the mouse; keratinocytes,³⁴ mesenchymal cells,³⁵ peripheral blood cells,³⁶ and adipose stem cells³⁷ in the human; and melanocytes in both species.³⁸ Among these, mouse stomach and liver cells showed reactivation of the ESC-specific Fbx15 gene during reprogramming much faster and contained fewer viral integrations than fibroblasts,²⁰ while human keratinocytes showed a capacity to be reprogrammed faster and more efficiently than human fibroblasts.^{16,34} These studies suggest that human cell types expressing the coxsackie virus and adenovirus receptor are well suited to adenoviral reprogramming. It is also likely that mouse hepatocytes are more easily reprogrammed than fibroblasts because their epigenetic and transcriptional state is similar to that of ESCs.²⁰ In addition, a recent publication made it clear that residual gene expression of the donor cell type contributes significantly to the differences between human iPS cells and ESCs. Thus, specific core sets of donor genes that continue to be expressed in each iPS cell line could lead to an incomplete reprogramming and could be a potential pitfall,³⁹ and whether the generation of iPS cells from adult hepatocytes will facilitate hepatic differentiation by transferring cellular memory is not known.

II.A. ESCs Versus iPS Cells

ESCs have already been successfully differentiated in vitro into various therapeutically relevant cell types, including motor and dopaminergic neurons, oligodendrocytes, cardiomyocytes, and hematopoietic precursor cells.⁴⁰ Of more significance, the therapeutic potential of these ESC-derived somatic cells has been effectively demonstrated in animal models of irradiated mice,²¹ blind mice,⁴¹ and a rat model of Parkinson's disease.^{42,43} The development of cell-replacement therapies using ESC differentiated cells is, however, burdened with social and religious concerns regarding the use of human embryos, as well as issues involving immune rejection of transplanted cells. Thus, iPS cells, which are derived from the patients themselves, are an ideal means with which to surmount these barriers.

A number of studies have demonstrated that iPS cells are similar in many ways to ESCs.^{21,44-50} In addition, functional differences between iPS cells and ESCs in terms of global gene expression levels and epigenetic state have also become clear.³⁹ Most iPS cell lines that have been generated thus far have a normal karyotype and possess telomeres with characteristics similar to those in ESCs.⁵¹ In addition, the ability of mouse iPS cells to generate an entire mouse was recently shown via tetraploid complementation assays (a technique in which iPS cells are injected into tetraploid blastocysts),⁵²⁻⁵⁴ and the formation of teratomas in vivo using human iPS cells³⁵ suggests that the different reprogramming approaches produce cells with a developmental potential similar to that of ESCs.

However, iPS long-term cell culture stability has yet to be investigated, and human ESCs grown in long-term cultures show genetic instability.⁵⁵ A recent study also reported that iPS cells can be used to efficiently generate hepatocyte-like cells. Although it was noted that subtle differences in the timing of onset and level of expression of different hepatic genes were found, the generation of hepatocyte-like cells from human iPS cells appeared to be as efficient as that observed from human ESCs.⁵⁶ Therefore, iPS cells have brought the realization of personalized regenerative medicine a little closer.

Recently, the potential of iPS cell-based cell replacement therapy in a humanized mouse model of sickle cell anemia has been published.⁵⁷ A similar approach was taken with human individuals with Fanconi anemia, a disease characterized by severe genetic instability.⁵⁸ In addition, iPS cell-derived dopamine neurons functionally have been integrated into adult brain in a rat model of Parkinson's disease, leading to an improvement of the phenotype.⁵⁹

Moreover, iPS cell-derived endothelial cells were introduced into the livers of hemophilia A mice, which resulted in survival in a death-inducing bleeding assay.⁶⁰

III. DIFFERENTIATION TOWARD HEPATOCYTES FROM ES/iPS CELLS

The generation of hepatocytes from iPS cells is a particularly appealing goal, because this parenchymal cell of the liver is associated with several congenital diseases.⁶¹ It is also the site of xenobiotic control, and is the target of many pathogens that cause severe liver dysfunction, such as the hepatitis B and C viruses. Moreover, unlike most other organs, the introduction of exogenous hepatocytes into the liver parenchyma is a relatively simple undertaking, suggesting that the liver is highly amenable to tissue therapy using iPS cell-derived hepatocytes.⁶²⁻⁶⁴

The ability to use primary hepatocytes either for therapeutic purposes or for basic research has been frustrated by their tendency to rapidly de-differentiate and lose most hepatic functions after growth in a tissue-culture environment.⁶⁵ It has therefore been necessary to find a readily available source of hepatocytes that can be grown for longer periods of time in culture. The need to expand primary hepatocytes purified from donor livers could be avoided by using stem cells to produce hepatocytes. The appeal of using iPS cells is that they could provide a source of autologous hepatocytes. The generation of hepatocyte-like cells from human iPS cells so far appears to be as efficient as that observed from human ESCs, although there are subtle differences in the timing of onset and level of expression of different hepatic genes.

Several studies have described the differentiation of human ESCs into cells that display hepatic characteristics. Initial models for deriving hepatocyte-like cells (HLCs) from human ESCs employed direct differentiation⁶⁶⁻⁷¹ or multicellular aggregate strategies.⁷²⁻⁷⁴ Direct differentiation approaches use a two-dimensional tissue culture approach employing extracellular matrixes, growth factors, cytokines, and hormones. The aggregation of human ESCs results in the formation of three-dimensional structures termed embryoid bodies. The culture of embryoid bodies on adherent matrixes in the presence of growth factors, cytokines, and hormone cocktails gave rise to varying levels of HLCs. To purify HLCs from a heterogeneous population, elegant experiments by Basma et al.⁷⁴ generated human HLCs through embryoid bodies, and purified these populations using fluorescence-activated cell sorting for the asialoglycoprotein receptor. The resulting cell population displayed hepatocellular function in many ways comparable to primary hepatocytes. Most recently, Si-Tayeb et al. demonstrated that mouse iPS cells can be induced to efficiently generate intact fetal livers, and that human iPS cells can be induced in culture to produce highly differentiated hepatocytes comparable to ESC-derived hepatocytes. Their study also showed the feasibility of generating cells with hepatic characteristics from skin cells through an iPS cell intermediate, and that such cells can at least engraft for a short time [AQ: 1] into the mammalian liver parenchyma.⁵⁶ Such proof-of-concept opens up the possibility of producing patient-specific hepatocytes in a relatively simple and straightforward manner with high efficiency. We are confident that such cells will become immediately useful for studies of hepatocellular disease and basic developmental mechanisms, as well as for drug-screening purposes.

IV. ORGAN-ENGINEERING TECHNOLOGY TO FACILITATE ESC/iPS CELL-DERIVED LIVER-TISSUE DEVELOPMENT

One important factor that is characteristic of in vivo tissue development using stem cells is the formation of elaborate three-dimensional structures. The structure of a tissue will, in most circumstances, incorporate a complex extracellular matrix and an intricate vasculature.

It has been shown that the efficient function of multiple cell types, including hepatocytes and islet hormone-producing cells, is dependent on matrix-producing cells and endothelial cells that provide a three-dimensional support structure and sufficient vascularization.⁷⁵⁻⁷⁷ Therefore, the three-dimensional interactions between numerous cell types play an important role in the function of tissue and may also be required for the efficient production of surrogate tissue structures derived from ESCs or iPS cells. Indeed, it has been observed that the process of differentiation of human ESCs into hepatocyte-like cells within three-dimensional collagen scaffolds gives rise to cells displaying morphological features, gene expression patterns, and metabolic activities characteristic of hepatocytes.^{73,78} Human ESCs differentiated within the three-dimensional environment of bioengineered scaffolds are also known to form complex tissue structures containing cells derived from all three somatic germ lineages.⁷⁹

Engineering of sophisticated, three-dimensional cell organizations (e.g., liver) is especially challenging. The liver is composed of several distinct cell types, including hepatocytes, sinusoid endothelial cells, Kupffer cells, stellate cells, and biliary epithelial cells. These cells are orchestrated through tight junctions and connective tissue such as type I and IV collagen and laminins.⁸⁰ In addition, a natural vascular tree with centralized inlet and outlet vessels combined with a pervasive exchange of nutrients supports these cells.

A recent paper by Ott et al.⁸¹ reported that whole-heart scaffolds with intact three-dimensional geometry and vasculature were obtained by decellularization of cadaveric hearts by coronary perfusion with detergents. Natural scaffold could then be repopulated with neonatal cardiac cells or rat aortic endothelial cells and cultured under simulated physiological conditions for organ maturation. Even though the actual contractile function of the recellularized construct was almost 2% compared with the normal contractile function of a normal heart, this work represents an example of organ engineering using decellularized natural scaffolds.⁸¹ Using the same technique, our group developed a decellularized whole-liver matrix sufficiently recellularized by primary hepatocytes, which demonstrates hepatocyte function comparable to optimized in vitro culture systems.⁸² This organ-engineering approach may provide a new platform for ESCs/iPS cells to generate liver tissue with disease-specific cells to model the natural liver environment.

The liver extracellular matrix presents an ideal scaffold for stem-cell differentiation into hepatocytes.^{83,84} It is known that local environmental factors induce hepatocyte homing, differentiation, and proliferation, and studies indicate that stem cells may differentiate toward mature hepatocytes following transfer into an injured liver.⁸⁵ Therefore, the decellularized liver matrix has significant potential as the scaffold for hepatocyte maturation. This process may be further promoted by the sequential delivery of factors involved in the initiation and maturation of stem cells to liver cells,⁸⁵ allowing temporal and spatial control over differentiation (Fig. 1).

V. MODELING LIVER DISEASES USING iPS CELLS

Further work needs to be done before attempting to generate clinically relevant iPS cells for human cell-replacement therapies; however, disease modeling and drug screening are two immediate applications of the reprogramming technology and the resulting iPS-differentiated cells.

The development of a human liver tissue model for the study of liver disease has been challenging, because human hepatocytes lose their characteristic function in in vitro cultures.¹⁰ Indeed, there are just a few appropriate in vitro disease models^{86,87} and in vivo chimeric disease models that have been developed using human hepatocytes, and these have been generated using complex techniques.⁸⁸ Animal models that are available have been

crucial in the investigation of disease mechanisms. However, fundamental developmental and physiological differences exist between human and mice models of liver. Thus, the importance of using human cells for these purposes is evident by a large number of failed clinical trials, which in part may be attributable to differences between species.⁸⁹

ESC or iPS cell technology offers an opportunity to develop functionally differentiated hepatocytes for in vitro analysis. If a functional and homogeneous hepatocyte-like cell population can be produced from stem cells by deriving cultures of known-specific genotypes, it may be possible to examine mechanisms responsible for genetic predisposition to disease. The added value emanates in having a human experimental platform as opposed to an animal model. The creation of disease-specific iPS cells allows experiments on the disease phenotype and offers an opportunity to repair the relevant gene defect. The iPS cell system is therefore a powerful stem cell modeling tool with which to derive patient-specific or disease-model cell types. With translation of human ESC models to iPS cell systems, it may also be possible to generate the desired cell types in sufficient quantity and scale for downstream applications.

The concept of utilizing ESCs, and now iPS cells, to model a disease in a culture dish is based on the unique capacity of these cells to continuously self-renew and their potential to give rise to all cell types in the human body.^{40,90} Thus, pluripotent cells could potentially serve as a limitless reservoir of cell types that, in many cases, were not previously possible to obtain.

During the recent past, there have been studies reporting successful in vitro modeling of diseases through either the overexpression of known disease-causing genes, such as SOD1 (superoxide dismutase 1) in the case of amyotrophic lateral sclerosis^{91,92} and NURR1 (nuclear receptor-related 1) in the case of Parkinson's disease,⁹³ or the derivation of ESC lines from pre-implantation embryos genetically diagnosed to be harboring mutations causing diseases such as fragile X syndrome.⁹⁴ Another example of in vitro modeling disease in the field of liver is VLCAD (very long chain acyl-CoA dehydrogenase) deficiency, an often fatal neonatal form of liver disorder due to mitochondrial beta-oxidation of fatty acids. It has been very complex to synthesize the enzyme in vitro; however, lately it has been expressed and purified using a bacterial system, giving a significant advance in our understanding of both the clinical aspects of VLCAD deficiency and the basic biochemistry of the enzyme.⁹⁵

The overwhelming advantage of iPS cell technology is that it allows for the generation of pluripotent cells from any individual in the context of his or her own particular genetic identity, including individuals with sporadic forms of disease and those affected by complex multifactorial diseases of unknown genetic identity, such as autism spectrum disorders,⁹⁶ type 1 diabetes, and liver enzymatic deficiencies.⁹⁷

There is an urgent need to develop liver-disease models to understand the complexity of the mechanism, including drug-induced liver injury,⁹⁸ enzymatic liver disease,⁹⁹ or chronic liver injury induced by HCV hepatitis.¹⁰⁰ Drug-induced liver injury has become a leading cause of severe liver disease in Western countries, and therefore poses a major clinical and regulatory challenge.¹⁰¹ Whereas previously, drug-specific pathways leading to initial injury of liver cells were the main focus of mechanistic research and classifications, current opinion is that these are initial upstream events. Subsequent common downstream pathways, and their attenuation by drugs and other environmental and genetic factors, also have a profound impact on the risk of an individual patient to develop overt liver disease.⁹⁸ Like environmental factors, genetic variations can principally affect all mechanisms involved in drug-induced liver injury, from drug metabolism over initial injury and mitochondrial

impairment to cell death and regeneration. Human iPS-derived hepatocytes under certain structural conditions can therefore facilitate the mechanism of hypersensitivity to drugs according to genetic polymorphisms^{102,103} or allergic hepatotoxicity.¹⁰⁴

Although many different types of disease models for metabolic disorders or hepatic virus infection are currently available, obtaining engraftment of human hepatocyte preparations in animal models that are applicable for the study of human disease has been particularly challenging.^{88,105} Hepatocyte transplantation in animal models of metabolic disorders, including the Gunn rat, a model of Crigler-Najjar syndrome type 1¹⁰⁶; Watanabe heritable hyperlipidemic rabbit, a model of human hypercholesterolemia¹⁰⁷; toxic milk mouse and Long-Evans cinnamon rat, a model of Wilson's disease^{108,109}; and a Dalmatian dog model of hyperuricosuria,¹¹⁰ have provided a foundation for clinical trials of hepatocyte transplantation in humans. Although these models are feasible for understanding the efficacy of clinical cell therapy, they are not suitable for understanding the mechanism of disease progression, nor do they consider the patient's specific background. Another challenge to in vitro disease modeling is chronic liver injury. Because fibrosis, and especially cirrhosis, are the major predictors of liver-related morbidity and mortality, there is an urgent need to develop, test, and monitor antifibrotic treatments that can prevent, halt, or even reverse liver fibrosis or cirrhosis. Fibrosis is an excessive wound-healing response that occurs in most forms of chronic liver disease and results in the deposition of scar tissue (i.e., excess extracellular matrix). With ongoing liver damage, fibrosis may progress to cirrhosis, which is characterized by a distortion of the liver vasculature and architecture. These factors are major determinants of morbidity and mortality in patients with liver disease, predisposing them to liver failure and primary liver cancer.¹¹¹ The usefulness of in vitro models such as cultured-activated hepatic stellate cells and hepatic stellate cell lines for drug discovery is limited because they do not reflect the complex interactions that orchestrate fibrogenesis or fibrolysis in vivo.

The following are the requirements that may be necessary for successful disease modeling in vitro: i) contributions of other cell types, or interactions between them; ii) the interaction of different cytokines, growth factors, and other mediators that are produced by different cells; iii) the use of normal or altered extracellular matrix; and iv) the changes in vascular architecture, oxygen supply, and production of reactive oxygen species. To address these requirements, cultured slices from normal and fibrotic liver were suggested as a tool for studying fibrogenesis.¹¹² More recently, a controlled three-dimensional co-culture system using primary hepatocytes and 3T3 fibroblasts has been reported.¹¹³

Projects that create multicellular, artificial co-cultures of hepatocytes, hepatic stellate cells, endothelial cells, Kupffer cells, and cholangiocytes are under way and may provide a highly reproducible and cost-efficient platform with which to develop and test antifibrotic drugs in a nearly physiological hepatic microenvironment. However, there is still the limitation of using primary cells because these cells cannot fully recapitulate the disease condition. Thus, if iPS cell- or ESC-based technologies can be combined with engineering approaches of three-dimensional structural construction, such as using the decellularized liver described above, it could provide disease-specific models generated from patients under stable conditions, which could address the current lack of humanized disease models.

VI. CONCLUSION

iPS cell technology has opened exciting avenues for human liver-disease modeling. Moreover, extensive characterization of the functionality of iPS cell-derived hepatic cells and their functional equivalence with in vivo counterparts needs to be extensively investigated and remains to be formally demonstrated. The application of the benefits that

iPS cells offer is also limited by the ability to derive disease-relevant somatic cells, and major challenges remain in defining pathways that efficiently lead to pure and functional populations of many liver disease-relevant cells. Even recapitulating disease in vitro has been a challenging task due to the lack of technology to induce fully differentiated cells. We are hopeful that the combination of iPS cell technology and liver-tissue engineering, and the creation of practical animal models of liver repopulation will have a positive impact on future therapeutic interventions, and may represent another arrow in the medical scientist's quiver that can be used to devise and test novel therapies for liver diseases.

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ABBREVIATIONS

ESC	embryonic stem cell
HLC	hepatocyte-like cell
iPS	induced pluripotent stem

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FIGURE 1.

Schematic representation of organ engineering for liver-disease modeling and transplantation. Whole-organ scaffolds serve as a template for cell delivery to re-engineer the sophisticated liver organotypic structures by using different types of liver cells, including hepatocytes, endothelial cells, cholangiocytes, stellate cells, neural cells, and Kupffer cells. (a) Decellularized rat liver; (b) transplantation of engineered auxiliary liver graft in the rat.