

# Future perspective of induced pluripotent stem cells for diagnosis, drug screening and treatment of human diseases

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## Summary

Recent advances in stem cell biology have transformed the understanding of cell physiology and developmental biology such that it can now play a more prominent role in the clinical application of stem cell and regenerative medicine. Success in the generation of human induced pluripotent stem cells (iPS) as well as related emerging technology on the iPS platform provide great promise in the development of regenerative medicine. Human iPS cells show almost identical properties to human embryonic stem cells (ESC) in pluripotency, but avoid many of their limitations of use. In addition, investigations into reprogramming

of somatic cells to pluripotent stem cells facilitate a deeper understanding of human stem cell biology. The iPS cell technology has offered a unique platform for studying the pathogenesis of human disease, pharmacological and toxicological testing, and cell-based therapy. Nevertheless, significant challenges remain to be overcome before the promise of human iPS cell technology can be realised.

## Keywords

Human induced pluripotent stem cells, reprogramming, *in vitro* disease models, drug screening

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## Introduction

The initial aim of stem cell biology is to provide insight into how genetic information is related to tissue and organ formation. Recent advances in this field nonetheless also show great promise in the development of regenerative medicine since many types of pluripotent stem cell can give rise to differentiated progeny *in vitro* and tissue formation in the human body. In addition, stem cell platforms that employ cell lineages derived from human stem cells represent a novel approach for drug development and screening (1). Human embryonic stem cells (ESC) derived from early blastocysts are the prototype of pluripotent stem cells that are capable of unlimited growth in tissue culture and can differentiate into all cell types in the body. As a result, human ESCs lines have been generated and used to study the relationships between gene function and tissue formation and organogenesis (2). Human ESC-derived specific cell types have also been explored for cell-based therapies for tissue regeneration as well as cell-based assays for drug screening (3).

The development of a human ESC platform is limited by the low efficacy in establishing human ESC lines, especially patient-specific ESCs via somatic cell nuclear transfer, the moral and ethi-

cal issues related to the use of human oocytes, as well as destruction of human blastocysts, and the immune rejection with allogeneic transplantation. One of the major recent breakthroughs in the field of stem cell biology has been the reprogramming of somatic cells into pluripotent cells by ectopic expression of transcription factors. In 2006, Takahashi and Yamanaka first reported that forced expression of four transcription factors: octamer 3/4 (Oct4), SRY box-containing gene 2 (Sox-2), Kruppel-like factor 4 (Klf4) and c-Myc out of an initial 24 factors screened, could reprogram mouse somatic fibroblasts into ESC-like colonies. These were termed induced pluripotent stem (iPS) cells (4). In 2007, Takahashi et al. and two other groups announced almost simultaneously their success in human iPS cell generation (5–7). Further enhancement of the technique, such as the use of Nanog and Oct4, rather than F-box protein (Fbx15) as selection have made human iPS cells to display almost identical properties to ESCs in terms of multi-lineage *in vitro* differentiation, teratoma formation, germline transmission, and even contribution to entire animals (8, 9). Nevertheless, there remain differences in gene expression patterns between iPS and ESCs (10). The generation of human iPS cells has circumvented the limitations of using human ESCs as discussed above. Thus iPS cell technology has opened up new avenues in biomedical research. In

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this review, the potential application of a patient-specific iPS cells as a platform for disease modelling and drug screening, as well as cell and tissue replacement therapies will be discussed (► Fig. 1).

## Recent advances in techniques to create iPS cells

The details of different methodologies to create iPS cells are beyond the scope of this review (11), thus it will confine itself to recent important advances. In addition to the initial use of fibroblasts for reprogramming, the use of various other somatic cell types, including melanocytes, mesenchymal cells, peripheral blood cells, and adipose stem cells, has been described (12). The different cell types used for reprogramming might lead to in variation of efficacy of reprogramming and differentiation potential. Among the different protocols used for reprogramming, Oct4 is believed to be the essential component and the other transcription factors can be compensated by the endogenous expression by initial cell types (12) or exogenous supplement of small molecules, such as DNA methyltransferase inhibitors and the histone deacetylase inhibitor valproic acid (12, 13). The use of genome-integrating viral vectors such as retroviruses and lentiviruses for reprogramming is associated with altered gene expression (14), and a potential risk of reactivation of viral transgenes. As a result, such iPS cells are inappropriate for therapeutic use: they may affect the phenotypes of their derived cells for disease modelling. Nevertheless, recent studies reveal that genome-integrating viral vectors can be removed using

the Cre-lox system (13, 14). Other techniques that do not require genome-integrating viral vectors, such as adenoviral, episomal and sendai virus vectors, repeated plasmid transfection, arginine peptide tagged proteins and small molecules, have also been reported (12–14).

## Disease modelling

Animal models can provide useful information about disease mechanisms, but they are limited by their fundamental differences in genetic background, physiology and pathophysiology compared with humans. Nonetheless it is unreasonable to use cell biology techniques for the investigation of human disease mechanisms using human tissue as in general this requires large amounts of live human cells and tissues from affected patients. Thus, the use of human stem cell platforms to derive different cells and tissues in the human body for disease modelling is an attractive option.

The promise of using ESCs, and more recently iPS cells, to model human disease is based on the unique capacity of these cells to continuously self-renew and differentiate into all cell types in the body. By capturing the entire genetic repertoire of an individual with a disease, one may be able to duplicate the pathogenesis of that individual's disease 'in a dish'. In order to apply a human ESC platform to disease modelling, over-expression of known disease-causing genes or a derived ESC line from pre-implantation embryos with a known genetic mutation are needed. This method is thus confined to diseases with a documented genetic defect.

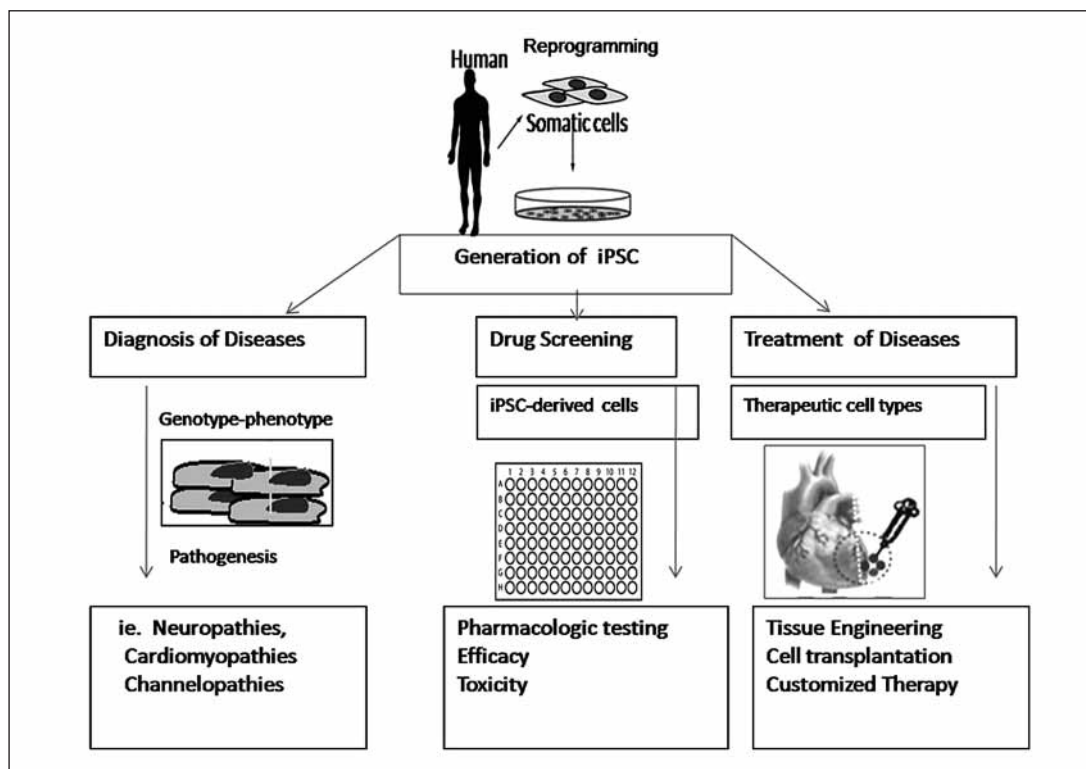


Figure 1: Potential clinical application of induced pluripotent stem cells for diagnosis, drug screening and treatment of human diseases.

The overwhelming advantage of iPS cell technology over ES cells is that patient-specific iPS cells are easily generated (15, 16). This is an important benefit given that many genetic diseases are of a sporadic nature with no family history. More than 10 human disease-specific iPS cell lines have been established, ranging from simple single gene deficiencies to complex multifactorial diseases of unknown genetic origin, such as type 1 diabetes (12, 13, 15). In addition, iPS technology will offer the unprecedented possibility of using human cells to study human diseases for which there are no animal models, such as Brugada syndrome and hypertrophic cardiomyopathy.

Recent studies have shown that patient-specific iPS cells can be used for human disease modelling. In 2009, Ebert et al. (17) reported the successful generation of iPS cells from a child with a genetic form of spinal muscular atrophy. These cells maintained the disease genotype, and were able to differentiate into motor neurons that showed selective deficits in survival of motor neuron protein aggregates, and were similar to the phenotype associated with motor neuron disease. Subsequently, Lee et al. have successfully generated patient-specific iPS cells from patients with familial dysautonomia, a neuropathy caused by a point mutation in the IκB kinase complex-associated protein (*IKBKAP*) gene (18). In addition to demonstrating the tissue-specific splicing defect in such iPS cell-derived tissue, the specific defects of the nervous systems were also duplicated using this technique. In both studies, the patient-specific iPS cell platform was used to provide new insight into the disease itself as well as potential new targeted treatment. Agarwal et al. (19) demonstrated that patient-specific iPS cells with dyskeratosis congenita could provide novel insight into the disease pathology. Patients with dyskeratosis congenita suffer from degeneration in multiple tissues due to disordered telomere maintenance. Reprogramming human somatic cells in these patients leads to telomere elongation and consequent correction of the defective telomerase RNA component in this disorder. In addition, these findings suggest that strategies to reverse this defective telomerase RNA component may offer a potential new therapeutic approach for patients with dyskeratosis congenita.

There remain nonetheless several major challenges to the use of iPS technology in modelling more complex diseases (12, 13, 15). First, late-onset human diseases have a long latency before the phenotypes can be manifested in the culture system. Therefore different approaches, such as exposure to oxidative stress and hypoxia in the cell culture system, have been proposed to accelerate the pathological phenotypes and reveal the potential susceptibility to these environmental stresses. Second, some of the disease processes may not be manifested by a single purified lineage-committed cell type. As a result, more than one cell type may be required during the co-culture assay. Third, diseases with a potential epigenetic contribution, such as those caused by combined genetic and environmental factors, may be difficult to study since the reprogramming process during generation of iPS cells should, in principle, remove any epigenetic changes. Overall though, iPS technology expands the horizon for studying pathogenesis in a culture dish. It will not completely replace current tools, such as transgenic animal models to study more complex diseases as well as their *in vivo* aspect.

## Drug screening

Current drug discovery and development programs are inefficient. Currently, more than 90% of lead candidates identified by the current *in vitro* screening systems fail to become drugs due to safety and efficacy issues in clinical applications. Although genetically modified rodents, immortalised human cell lines and animal models have provide useful information in studying safety and efficacy of drugs, their major weakness is that they fail to replicate human conditions. The treatment response to drugs in animal models cannot be used to predict efficacy in humans. For example, creatine (20) is very effective in altering the disease characteristics of amyotrophic lateral sclerosis due to over-expression of mutant superoxide dismutase in a transgenic mouse model. Nonetheless, no clinical improvements have been observed in human clinical trials (21). Individual variability in response to potential therapeutic agents also cannot be tested using the current drug testing platform (12, 22). Additional drug screening model systems are thus needed to better mimic human conditions (16).

The iPS cell technology may provide a novel approach to pharmacological and toxicological testing (12, 13, 16). First, the iPS cell platform allows the generation of human disease-specific cell types to enable better prediction of therapeutic response and toxicology, for example neurons, cardiomyocytes, and hepatocytes. Second, a library of different iPS cell lines for the same human disease can be generated and provide insight into the genetic and potentially epigenetic variation of a broad section of the population. Third, the variation in therapeutic effect of a potential drug can be tested at an individual level. Personalised medicine thus becomes a reality with the use of patient-specific iPS cells.

Cardiac and liver toxicity are a major cause of drug failure during pre-clinical and clinical testing. The lack of an *in vitro* model to detect pro-arrhythmic effects of drugs on human heart cells has hindered the development of many therapeutic agents: iPS cell-derived cardiomyocytes (23, 24) can provide a valuable cell source to test drug efficacy and safety prior to clinical testing. The use of iPS cell-derived cardiomyocytes from patients with long-QT syndrome may be used as a model to test the safety of potential candidate agents against this lethal arrhythmia. Treatment response of many genetic disorders known to affect heart function, such as dilated cardiomyopathy, can also be determined with this iPS cell platform. Recent studies have validated this concept using human iPS cell-derived cardiomyocytes together with a multi-electrode assay as a platform to study the changes in electrophysiological properties of heart cells with different agents (25, 26).

Disease- or patient-specific iPS cells thus have great potential in pharmaceutical development. The iPS cell technology can be applied in high-throughput screening assays and also used in prediction of individual patient response to therapeutic agents. In the early-stages of drug development, the use of iPS cell technology can substantially minimise the number of animals sacrificed during drug testing, enable early human toxicity to be detected in pre-clinical trials, and decrease the risk and cost associated with clinical trials. While iPS technology provides great opportunities for drug screening and toxicity testing, we should be aware that cells

generated from iPS cells are developmentally immature, and may better represent a model for fetal biology. In order to reflect adult human biology, maturation of the iPS-derived cells is required prior to their use for drug development. The provision of lineage specific reporter lines through genetic manipulation to drive the differentiation and maturation of iPS cells to specific cell types should enhance this development.

## Cell-based therapy

Following the success of hematopoietic stem cell therapy in the treatment of haematological diseases, the potential application of cell based therapy has been extended to the treatment of other human diseases. In particular, different types of adult stem cells, including bone marrow, peripheral haematopoietic, and mesenchymal stem cells (MSC) have been evaluated in the treatment of cardiovascular disease (► Table 1) (27–29). Nonetheless adult stem cells have limited potential for proliferation and differentiation, thus ESCs have been explored for tissue regeneration as they can be differentiated into various therapeutic relevant cell types *in vitro* (30). Despite this, there is limited progress in the use of ESCs

for tissue regeneration in humans due to various technical, social and religious issues (12).

The generation of patient-specific iPS cells has the advantage of avoiding many of the ethical concerns associated with the use of embryonic or fetal material, and have no risk of immune rejection. Currently, several therapeutic relevant cell types, including motor neuron (31), hepatocytes (32, 33), pancreatic insulin producing cells (34), haematopoietic cells (35–37), retinal cells (38), cardiomyocyte (39, 40) and MSCs (41), have been successfully derived from human iPS cells, and some of them have been tested to treat diseases in animal models. In murine models of haemophilia A and sickle cell anaemia, iPS cell-based therapies have been shown to attenuate the disease process (35, 36). The use of iPS cells has thus been proposed as diagnostic and therapeutic tools for different haematological disorders (37). Carr et al. (38) have demonstrated that transplantation of human iPS cell-derived retinal cells can rescue retinal dysfunction in rats. This suggests that retinal cells derived from healthy iPS cells are functional, and can potentially be used to treat retinal dysfunction. For cardiovascular regeneration, different iPS cell-derived cell types, including cardiomyocytes (23, 40) or MSCs (41) have been successfully generated. Nelson et al. (39) first reported the use of iPS cells for myocardial repair in animal models of acute myocardial infarction. Lian et al. (41) demon-

Cell types	Advantage	Disadvantage
Embryonic stem cells	<ul style="list-style-type: none"> <li>● Pluripotent and unlimited supply</li> <li>● Patient-specific cells for autologous transplantation possible via therapeutic cloning</li> </ul>	<ul style="list-style-type: none"> <li>● Social and ethical concerns</li> <li>● Risk of rejection and required immunosuppression for allogenic transplant</li> <li>● Limited supply of human oocytes</li> <li>● Risk of tumor formation</li> <li>● Proarrhythmic risk due to immature phenotype of derived cardiomyocyte</li> </ul>
Induced pluripotent stem cells	<ul style="list-style-type: none"> <li>● Pluripotent and unlimited supply</li> <li>● Patient-specific cells for autologous transplantation possible</li> </ul>	<ul style="list-style-type: none"> <li>● Risk of tumor formation</li> <li>● Risk of viral vector</li> <li>● Proarrhythmic risk due to immature phenotype of derived cardiomyocyte</li> </ul>
Skeletal myoblast	<ul style="list-style-type: none"> <li>● Autologous transplantation without the need for immunosuppression or risk of rejection</li> <li>● Can be expanded <i>in vitro</i> with high yield, resistant to ischemia &amp; fatigue</li> </ul>	<ul style="list-style-type: none"> <li>● Cannot differentiate into cardiomyocyte phenotype</li> <li>● Lack of integration with host cardiomyocyte with arrhythmogenic potential</li> </ul>
Bone marrow stem cells	<ul style="list-style-type: none"> <li>● Autologous transplantation without the need for immunosuppression or risk of rejection</li> <li>● Can induce angiogenesis, possible pluripotent</li> </ul>	<ul style="list-style-type: none"> <li>● Limited ability to differentiate into cardiomyocyte</li> <li>● Limited supply and need for <i>in-vitro</i> expansion</li> <li>● Difficult to isolate and propagate in culture</li> </ul>
Mesenchymal stem cells	<ul style="list-style-type: none"> <li>● Autologous transplantation without the need for immunosuppression or risk of rejection</li> <li>● Can induce angiogenesis and possible pluripotent</li> <li>● Lower risk of rejection and ? possible for allogenic transplantation</li> </ul>	<ul style="list-style-type: none"> <li>● Limited ability to differentiate into cardiomyocyte</li> <li>● Limited supply and need for <i>in-vitro</i> expansion</li> <li>● Difficult to isolate and propagate in culture</li> </ul>
Adult cardiac stem cells	<ul style="list-style-type: none"> <li>● Cardiomyocyte phenotype with no need for differentiation</li> <li>● Can integrate with host cardiomyocyte</li> <li>● Autologous transplantation without the need for immunosuppression or risk of rejection</li> </ul>	<ul style="list-style-type: none"> <li>● Very limited supply</li> <li>● Difficult to isolate and propagate in culture</li> <li>● Proarrhythmic risk due to immature phenotype of derived cardiomyocyte</li> </ul>

**Table 1: Different types of stem cells for cardiovascular diseases.**

strated that human iPS cell-derived MSCs are superior to adult bone marrow-derived MSCs in the attenuation of hindlimb ischaemia in mice. Despite the similarities between phenotypes of MSCs derived from bone marrow and iPS cells, those derived from iPS cells had better survival and engraftment following transplantation to induce vascular and muscle regeneration via direct *de novo* vascular and muscle differentiation and paracrine mechanisms. Therefore, stem cells derived from pluripotent stem cells seem to have better therapeutic efficacy compared with those from adult stem cell sources.

There are several major challenges to overcome before iPS cell technology is applied in clinical practice. First, current iPS cells are not “clinical grade”. Genome-integrating viral vectors used for reprogramming are known oncogenes, particularly *c-Myc*, *Oct4* and *Klf4*, such that iPS cells thus generated are unlikely to be safe for clinical application. Nonetheless recent technological advances, including reprogramming without viral integration such as plasmids or direct reprogramming protein delivery assays can solve this issue (12, 13). Second, the efficiency of human iPS cell generation using classic retroviral-mediated reprogramming is as low as 0.001%–0.01%. Further improvement in iPS technologies, such as the inhibition of p53-mediated pathways (42, 43) and vitamin C supplementation (44), are needed to enhance the generation of iPS cells. Third, it remains unclear whether the human iPS cell clone has complete nuclear reprogramming. The stringent pluripotency state, as verified by the tetraploid complementation assay in mice (9), is not applicable to humans. Incomplete reprogramming of somatic cells to iPS cells could result in impaired differentiation of iPS cells into the required cell type (45). Finally, all pluripotent stem cells have the potential for teratoma formation. Stringent tests are thus required to ensure that all iPS cell-derived therapeutic cell types do not contain any undifferentiated iPS cells prior to transplantation. In the mouse model, teratoma formation can be seen with as few as 10,000 undifferentiated human ES cells (46).

In future, novel technologies must be developed to track cell fate *in vivo* for pre-clinical and clinical trials. Despite the challenges in the therapeutic use of iPS cells, preclinical studies have provided the proof-of-concept that patient-specific iPS cells can provide an unlimited cell source to produce massive therapeutic cell types, such as cardiomyocytes and MSCs, and can be prepared in an “off-the-shelf” format for cell transplantation.

## Future perspectives

A decade of studies in human ESCs has yielded remarkable progress and understanding in stem cell biology. The technical challenge of creating patient-specific ESCs, the ethical issues arising from the fetal origin of human ESCs and the potential risk of immune rejection make broad clinical application of this cell type difficult. Recent advances in human iPS cell technology can potentially circumvent these disadvantages: iPS cells thus provide an invaluable resource of cell types for modelling diseases, drug or toxicology screening, and patient-specific cell therapy. Significant

challenges remain to be overcome before the full potential of human iPS cell technology can be realised.

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