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Induced Pluripotent Stem Cells: Current and Emerging Technologies

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1. Introduction

Induced pluripotent stem (iPS) cells are somatic cells which have been imbued with pluripotent differentiation potential through some form of artificial treatment. On a general level, these treatments involve modifications in the expression of keystone genes associated with pluripotency in embryonic stem cells (ESCs) or their downstream expression products. Despite the conceptual simplicity of iPS cell technology, the 2006 development of the first iPS cell line by Yamanaka and Takahashi [1] has led to an exponential increase in the volume of pluripotency research and a new perspective from which to approach regenerative medicine.

iPS cells are a potential alternative to ESCs in therapeutic contexts, retaining the regenerative potential of ESCs inherent in pluripotent phenotypes, while bypassing some of the risks associated with ESC transplants. A number of studies have demonstrated that iPS cells and ESCs have effectively indistinguishable pluripotent capability, implying that iPS cells maintain the same therapeutic potential long associated with natural ESCs. However, unlike ESCs, iPS cells do not carry a risk of immunorejection due to their patient specific nature, and are not affected by the same ethical concerns as ESCs. As such, iPS cells may actually be preferable to ESCs in some therapeutic contexts due to reduced risk factors for the patient.

Since Yamanaka's hallmark 2006 paper and methodology, numerous iPS cell generation technologies have been developed. Most methods rely upon epigenetic expression of genes determined to be pluripotency regulators. Expression is most commonly induced through viral integration into the host genome, though other episomal methods do exist. Non-genetic induced pluripotency methods generally utilize the downstream expression products of the same keystone genes to generate the same effect as epigenetic expression, without requiring the host to transcribe and generate the products independently.

iPS cells promise a new paradigm in regenerative medicine. Developing iPS technologies have the potential to generate patient specific stem cells, for use in generating any target phenotype within the human body for transplant. In the research context as well, iPS cells have the potential to greatly advance existing disease models. Patient specific iPS cells could be used to create individualized disease models, potentially allowing for more specialized treatment of patients. Here, we discuss a number of the technologies in development seeking to fulfill these promises, as well as their potential applications in both therapeutic and research settings.

2. Canonical methodology

The seminal event in the development of iPS technology, Yamanaka and Takahashi's 2006 publication demonstrated for the first time that the pluripotent phenotype could be induced in somatic cells and was not exclusive to ESCs. In their initial approach, Yamanaka et al. screened 24 genes as potential candidates to induce pluripotency in mouse embryonic fibroblasts (MEFs). The candidate genes were chosen for their perceived roles in regulating pluripotency in ESC cultures. From the initial candidates, four genes were eventually identified to be necessary for induction of pluripotency, each shown to play a role in ESC pluripotency regulation: Sox2 [2], Oct4 [3], Klf4, and Myc-c [4], often abbreviated as SOKM. MEF cultures were transduced using four pMXs-based retroviral vectors, each containing one of the target transcription factors. MEFs transduced with these factors formed colonies exhibiting ESC morphology and the pluripotent phenotype, as demonstrated by their differentiation ability and teratoma formation *in vivo.*

Groundbreaking as it was, this initial iPS technology had multiple issues preventing immediate use in downstream applications. Despite its effectiveness, the early SOKM method had a decidedly low efficacy [1, 5], inhibiting generation of large scale iPS cultures for use in potential clinical applications or in the laboratory. The viral integration method also presented challenges, as genome integration could lead to random gene reactivation within the iPS culture, potentially causing deleterious effects. Myc-c itself acts as a protooncogene, which led to tumorogenesis in 50% of mice chimeric mice derived using the SOKM method [6]. Compounded with the risk for random gene reactivation, the use of Myc-c could lead to tumorogenesis in potential patients.

3. Improved epigenetic methods

In the interim since the development of the initial induction methodology, many improvements and variations on the technology have been made. Most of these improved methods utilize a similar epigenetic pathway to that of the original study, relying upon the host culture to express downstream products which induce the pluripotent phenotype. We discuss in brief some of these improved epigenetic methods, their potential niche applications, and their delivery vectors.

3.1. Sox2, Oct4, Lin28, Nanog

Yu et. al. demonstrated in 2007 that iPS cells could be generated from pre-natal and post-natal fibroblasts without transduction of the protooncogene Myc-c, using a combination of Sox2, Oct4, Lin28, and Nanog (SOLN). Factors were selected based on their high expression in ESCs, in comparison to myeloid progenitors. Removal of Myc-c from the gene cocktail eliminated the risk of transcription factor induced tumorogenesis, overcoming one of the fundamental issues with Yamanaka and Takahashi's initial methodology. Additionally, Yu et. al. recognized the potential usefulness of Nanog in iPS technologies, noting that it could lead to an increased recovery rate for iPS cell clones generated using the SOLN method. This is potentially due to Nanog's action upstream of Oct4 and Sox2. Lin28 did not integrate in one iPS clone from each of the two cell lines tested, suggesting that while Lin28 may improve efficacy, it is not necessary for reprogramming [7].

3.2. iPS-S: Sox2, Oct4, Lin28, Nanog, Klf4, Myc-c

Combining the SOLN and SOKM transcription factor cocktails, Liao et al demonstrated in 2008 that the efficacy of transfection could be improved by using all 6 previously demonstrated transcription factors in a single transduction and deemed their method iPS-S. Combination of the 6 factors was attempted based on empirical speculation, and proved successful. Transduced colonies also developed more rapidly, within 17 days post-transduction, as opposed to 26 days using the standard SOLN factors. The iPS-S method also increased efficacy roughly 10 fold, which combined with the more rapid development of iPS colonies, partially addressed the inefficiency issues with the canonical iPS technology [8]. As with other transcription factor combinations utilizing Myc-c, the iPS-S method carries with it a risk of tumorogenesis due to random transgene reactivation, inhibiting the use of iPS-S in some applications.

3.3. Combined epigentic, small-compound, and endogenous expression approaches

One of the first approaches to reduce the number of factors required, it was demonstrated that treatment with the epigenetic small compound BIX-01294 could substitute transduction of Sox2 or Oct4, using the traditional SOKM combination, in neural progenitor cells (NPCs) [5]. The study was notable for multiple reasons, both the use of chemical conditions to remove transcription factors, and the reliance upon endogenous gene expression in the target somatic cell line. While reliance upon Sox2 expression in NPCs ultimately limits the applicability of the BIX-OKM combination, it set a precedent for use of endogenous gene expressions to reduce the number of necessary transcription factors in certain cell lines, potentially allowing for safer, more efficient iPS generation in specific contexts. This concept was further explored by Kim et. al., who demonstrated that Oct4 alone was capable of inducing pluripotency in neural stem cells (NSCs) due to their endogenous expression of Sox2, Myc-c, and Klf4 [9].

Shi et al. improved upon their original small compound approach, eliminating the need for Myc-c transfection and endogenous Sox2 expression. BIX-01294 and non-genetic calcium channel agonist BayK8644 were identified via a phenotypic compound screen of known drugs, and combined with the transduction of Oct4 and Klf4 (OK), were able to induce pluripotency

in MEFs. The elimination of multiple transcription factors suggests that it may be possible to further replicate the effects of epigenetic transduction using chemical conditions, reducing the risk for random gene reactivation and potentially allowing for more controlled iPS generation temporality [10].

Year	Group	Vector	Transcription Factors
2006	Yamanaka et. al.	Retroviral	Sox2, Oct4, Klf4, Myc-c
2007	Yu et. al.	Lentiviral	Sox2, Oct4, LIN28, Nanog
2008	Shi et. al.	Retroviral, small-compound *	Oct4, Klf4, Myc-c, small-compound BIX-01294
2008	Shi et. al.	Retroviral, small-compound	Oct4, Klf4, small-compounds BIX-01294, BayK8644
2008	Liao et. al.	Lentiviral	Sox2, Oct4, Klf4, Myc-c, Lin28, Nanog
2008	Okita et. al.	Plasmid	Sox2, Oct4, Klf4, Myc-c
2009	Kim et. al.	Retroviral *	Oct4
2009	Fusaki et. al.	Sendai virus	Sox2, Oct4, Klf4, Myc-c
2010	Sugaya et. al.	Retroviral, plasmid	Nanog

Table 1. Epigenetic methods covered in this section: year of publication, vectors used, and required transcription factors. * Reliant upon endogenous expression of certain somatic cell phenotypes.

3.4. Nanog

While the majority of epigenetic approaches rely upon multiple transcription factors, chemical conditions, or endogenous expression, we patented technology capable of generating iPS cells through transfection of Nanog alone in 2006 [11]. Nanog is capable of inducing pluripotency without the aid of other factors due to its role upstream of Oct4 and Sox2. We demonstrated this interaction in bone marrow derived mesenchymal stem cells (MSCs), in which Nanog transfection successfully increased Sox2 and Oct4 levels [12]. Nanog has been demonstrated to induce pluripotency when delivered through lentiviral or plasmid vectors, providing both integrated and episomal gene expressions pathways.

Eliminating the need for multiple transcription factors has various benefits. As with other modified gene cocktails, the elimination of Myc-c greatly reduces the risk of tumorogenesis. Transfection of a single genetic factor may have higher efficacy than that of multiple factors and could lead to a lower overall cost per iPS cell generated. The improved efficiency and reduced cost of this method could allow for more rapid production of iPS cells for use in therapeutic treatments at a lower eventual cost to the patient.

3.5. Retroviral vectors

Beginning with the seminal paper by Yamanaka and Takahashi, the majority of improved epigenetic methods have utilized retroviral vectors to deliver their target transcription factors. In the context of induced pluripotency, retroviral vectors provide a number of distinct advantages, leading to their widespread use. Due to integration with the host genome, retroviral vectors are capable of generating stable iPS clones that maintain their phenotype

over time, unlike some episomal vectors. Retroviral technologies are very mature, allowing for rapid development of vectors and efficient production of vectors in the laboratory. While standard retroviral vectors are only capable of infecting dividing cells, the lentiviral subclass of retroviruses are indeed capable of infecting non-dividing cells, an important consideration when infecting cell types that divide rarely, such as neurons. A combination of these attributes makes retroviral vectors a highly functional candidate for iPS cell induction.

However, retroviral vectors and the lentiviral sub-class also have certain inherent risk factors. Most prominently, viral integration into the host genome can cause random gene reactivation, as discussed in section 2. Using the original SOKM transcription factors, this risk is exaggerated due to the protooncogenetic nature of Myc-c. Although various epigenetic methods have eliminated the necessity of Myc-c, random gene reactivation may still lead to tumorogenesis and deleterious effects in potential transplant patients. As a case study, an FDA clinical trial involving the retroviral transduction of non-protooncogenes led to the development of lymphoma in two patients [13]. Residual expression of transgenes may also lead to phenotypic expression differences between iPS cells and ESCs, leading to a less accurate model of human ESCs for research use or some clinical applications [7, 14]. To reduce the risk of random transgene reactivation and minimize remnant transgene expression, transgenes can be excised using a Cre/Lox system, as demonstrated by multiple groups [12, 14].

3.6. Plasmid vectors

Episomal factors, by definition, allow for the introduction of genetic factors without integration into the host genome. A lack of host genome integration inherently removes the risk of random transgene reactivation associated with viral vectors, but presents functional challenges in some contexts. The most common type of episomal vector in the context of iPS technology is the plasmid, a DNA library separate from the host's nucleic genome, first confirmed as a viable reprogramming vector using the original SOKM factors [15]. The plasmid method has several advantages, both in the laboratory and in downstream applications. Plasmids are a well-developed technology, are very easy to generate in great quantity in the laboratory, and have a relatively low cost-of-use compared to comparable viral vectors. For these reasons, plasmids are the favored vector in Yamanaka's laboratory [16].

The most prominent advantage of plasmids is the lack of integration inherent in episomal vectors. Although there is a potential for spontaneous integration of transgenes during the reprogramming process, iPS clones generated from plasmid vectors can be screened to select only integration-free clones [17]. As such, plasmid vectors are unaffected by issues related to transgene integration, such as residual transgene expression and random transgene reactivation. Although these advantages make plasmids a desirable vector for reprogramming, their efficacy remains well below that of viral integration, limiting the potential for large scale iPS cell generation using plasmids [18]. This reduced efficacy could potentially be due to the temporary nature of plasmids, and the speculated ongoing nature of the reprogramming process [18]; transcription factor expression may be reduced before the iPS reprogramming process is complete, altering the stoichiometric balance of factors and ending reprogramming

Vector	Advantages	Disadvantages
Retroviral	Genome integration allows single transduction iPS clone generation, well-developed technology, relatively efficient transduction rates	Genome integration may lead to random gene reactivation, cannot infect non-dividing cells, residual transgene expression concerns, can potentially induce immunogenicity
Lentiviral	Genome integration allows single transduction iPS clone generation, well-developed technology, relatively efficient transduction rates	Genome integration may lead to random gene reactivation, residual transgene expression concerns
Plasmid	Produces integration-free iPS clones, relatively low cost, volume production is easily scalable	Lower efficacy than viral integration methods, clones must be screened to check for integration
Sendai Virus	Produces integration-free iPS clones, relatively high efficacy	Pluripotent gene expression degrades over the course of 18-20 passages, clones must be screened for viral genome remnants

Table 2. Comparison of different epigenetic vector technologies

in cells that may have otherwise formed colonies. Depending upon the chosen transcription factor combination and somatic cell phenotype, plasmid transduction may also require multiple transfections to effectively reprogram cells, increasing the difficulty and labor-intensiveness of the technique.

3.7. Sendai virus

Sendai virus is widely known to replicate in the cytoplasm of host cells without integrating into the host genome. As such, it has been widely studied as an efficient expression vector and is known to effectively express transgene without integration [19-21]. Fusaki et. al. have demonstrated that a sendai virus vector carrying the four SOKM factors is sufficient to successfully generate iPS colonies [22]. Sendai virus reprogramming was shown to be as or more effective than traditional retroviral reprogramming, with a ~1% efficacy. Even though sendai viruses do not integrate into the genome, the persistence of a viral genome within iPS clones remains a concern for downstream applications. However, Fusaki et. al. were able to isolate clones that had no remnant presence of viral genomes. As such, the sendai viral vector is very attractive for use in downstream clinical applications. In the laboratory, sendai viral vectors leave something to be desired. Pluripotent gene expression of sendai induced iPS cells has been shown to degrade over the course of 18-20 passages, making long term iPS clone maintenance difficult.

4. Non-genetic reprograming methods

Complimenting research into genetic induction of pluripotency, a number of avenues into non-genetic iPS generation have been studied. Although non-integration epigenetic methods have been developed, many are inefficient, and cannot completely eliminate the possibility of

genome alteration. Non-genetic induction removes the risk of genetic factor reactivation and consequent genetic modification inherent with these epigenetic methods. To circumvent genetic transfection, technologies have been developed which utilize downstream RNA and protein phases of the desired genetic factors to induce expression.

4.1. mRNA transduction

Warren et. al. have demonstrated that modified mRNAs transcribing for the four SOKM factor proteins are capable of reprogramming when passed into the cytosol of various human cell types with a catatonic delivery vehicle [23]. Initially, cytotoxicity of transfected mRNAs inhibited effective reprogramming, requiring modifications to the mRNA. In a novel approach, Warren et. al. modified the ribonucleotide bases of vector mRNAs by substituting 5-methyl-cytidine for cytidine and pseudouridine for uridine, reducing the immunogenicity of the mRNAs [24]. Combined with interferon inhibitor media supplements, the modifications allowed for generation of viable iPS clones.

mRNA induced pluripotent stem (RiPS) cell generation is highly efficient relative to other technologies, with an efficacy of 1.34% in Warren's initial study. However, the modified mRNAs are difficult to generate in the laboratory and the techniques are labor-intensive. Repeated mRNA administrations are also required, increasing the labor-intensive nature of the technique and complicating volume production of RiPS clones.

4.2. Protein transduction

Multiple groups have also demonstrated reprogramming utilizing the protein products of the SOKM factors [25, 26]. In order for the target proteins to pass through a lipid bilayer, both groups attached each target protein to a cell penetrating peptide (CPP). At this stage, cells are treated with CPP-conjugated proteins multiple times to ensure a continuous supply of reprogramming factors. The protein induced pluripotent stem (piPS) cell induction method is significantly less efficient than epigenetic methods, with an efficacy of ~0.001%. In addition to the transduction inefficiencies, the temporality of the process is relatively slow and the multiple treatment protocols are very labor intensive, making volume production of piPS clones difficult. Although inefficient, the piPS method does eliminate the risk of transgene reactivation and genome integration, just as the RiPS method.

5. Optimization of induction methods

5.1. Factors affecting efficacy

While each induction method has an inherent relative efficacy, it must be noted that a number of external factors affect reprogramming efficacy as well. Multiple groups have reported that O_2 concentrations play a role in reprogramming efficacy [27, 28], with hypoxia noted to increase efficiency. The presence of methylation inhibitors, such as 5'-azacytidine, in culture medium have also been noted increase efficiency [29, 30]. Hanna et. al. have also demonstrated that cell

division rate plays a role in the kinetics of iPS induction [31]. Findings such as these suggest that the specific culture environment play a major role in pluripotency induction and may effect downstream development of iPS clones.

Of prominent concern, it has been reasoned that the stoichiometric abundances of reprogramming factors in relation to one another plays a role in reprogramming efficacy [18]. This rationale is based upon the differential effects of some pluripotency factors when expressed in different levels; for instance, expression of Oct4 and Sox2 at median levels can maintain pluripotency of ESCs, but overexpression of Oct4 can induce differentiation [32]. A similar action has been demonstrated in the context of iPS induction, in which a threefold increase of Oct4 increased efficacy, but further increases reduced the efficiency of reprogramming [33]. As such, the ability to monitor and manipulate the stoichiometric expression levels of transcription factors may play a role in selection of vectors and induction technologies in the future.

iPS cells have recently been shown to possess preferential differentiation based on their somatic cell origin, referred to as epigenetic memory [34-36]. It is believed that variations in DNA methylation status allow differentiation preferences to persist beyond the boundaries of reprogramming. It may be possible to exploit this epigenetic memory to increase the terminal differentiation efficiency of iPS cells based on the desired differentiated phenotype. By selecting cells of origin in the same lineage, or tissues known to have limited transdifferentiation ability into the target cell type, it may be possible to augment the efficacy of current induction protocols.

5.2. Application specific induced pluripotent stem cells

Until now, the majority of iPS cell research has sought to increase the efficacy at which stable pluripotent iPS clones could be developed. However, for the optimal production of a desired differentiated phenotype, solely optimizing the efficacy at which iPS clones can be developed may not be the best strategy. First touched on by Yamanaka in 2009 as the concept of "functional pluripotency" [6], it may be more effective to optimize for the generation of a target differentiated phenotype in the context of downstream applications. As such, a number of the efficacy factors mentioned above could be considered and optimized for each target phenotype and each downstream application.

Until such a time as reprogramming efficacy improves dramatically, the optimization of reprogramming in the context of specific downstream applications may be a way to increase efficiency. For each application, specific factors regulate the optimal induction method and environment, such as the acceptability of genome integration, the temporality in which desired phenotypes are needed, and the volume in which the target phenotype is required. Based on application specific factors such as these, it may be optimal to utilize various induction methods combined with an optimized set of efficacy conditions described above to generate iPS cell products on an application by application basis, rather than focusing solely on improving the generation of iPS clones.

6. Clinical applications

iPS cells can theoretically become any tissue in the body, which opens a number of possibilities for the use of iPS derived cells in graft and transplant based treatments. A key advantage of iPS cells is patient-specificity. iPS cells could be generated from a patient's own somatic cells and differentiated into the desired phenotype, allowing for an effectively autologous transplant which attenuates the risk of immunorejection. In the manner, iPS technology can be used as a pathway of sorts to generate desired tissues for transplant and tissue engineering applications (Figure 1).

6.1. Acute neurological damage

Induced pluripotent stem cell technologies have provided an exciting avenue for potential treatment of many neurological diseases, many of which have few treatment options at present. Among these disorders, acute neurological damage has an exceedingly direct treatment model through the iPS pathway. In many cases, such as stroke or spinal cord injury, direct transplantation of neuronal cells derived from patient-specific iPS cells to the damaged region could potentially aid in convalescence. Studies have already demonstrated functional recovery in spinal cord injury models of mice treated with iPS derived neuronal cells [37]. Groups have also confirmed functional recovery in peripheral nerve regions [38] and murine ischemia models [39, 40], with promising graft cell growth rates in ischemia models, and notable integration with existing neural networks. These findings show promise for the potential of iPS cell therapies in acute neurological damage conditions; however, further research is needed to ascertain the efficacy, safety, and long term effects of such transplantations.

6.2. Parkinson's disease

Some higher cognitive disorders could also be addressed using similar direct transplantation therapies. Parkinson's disease is perhaps the most direct of the higher cognitive disorders to address in this manner, as the primary cause of functional degradation can be traced to a single cell phenotype. The loss of dopamine secreting neurons in the *substantia nigra* region of the brain has been established as the leading cause of many Parkinson's symptoms, suggesting that direct replacement of lost dopamine secreting neurons through iPS cell derived neurons could aid in recovery. Through the use of various methods, multiple groups have efficiently differentiated iPS cells into dopaminergic neurons [41, 42], overcoming the first obstacle in the implementation of a transplantation therapy. In a rodent model, transplantation of dopaminergic neurons and other neuronal phenotypes into Parkinson's disease model were able to induce functional recovery [43].

These results demonstrate the potential of iPS cells to provide functional recovery in Parkinson's disease patients. However, further research is needed to establish the degree of recovery post-transplant, to improve the efficacy of transplantation, and to assess the long-term benefit of transplantation. It has been suggested that transplanted neuronal populations derived from iPS cells of hereditary Parkinson's patients may be inclined to exhibit similar degenerative phenotypes after implantation and this potential must be explored.
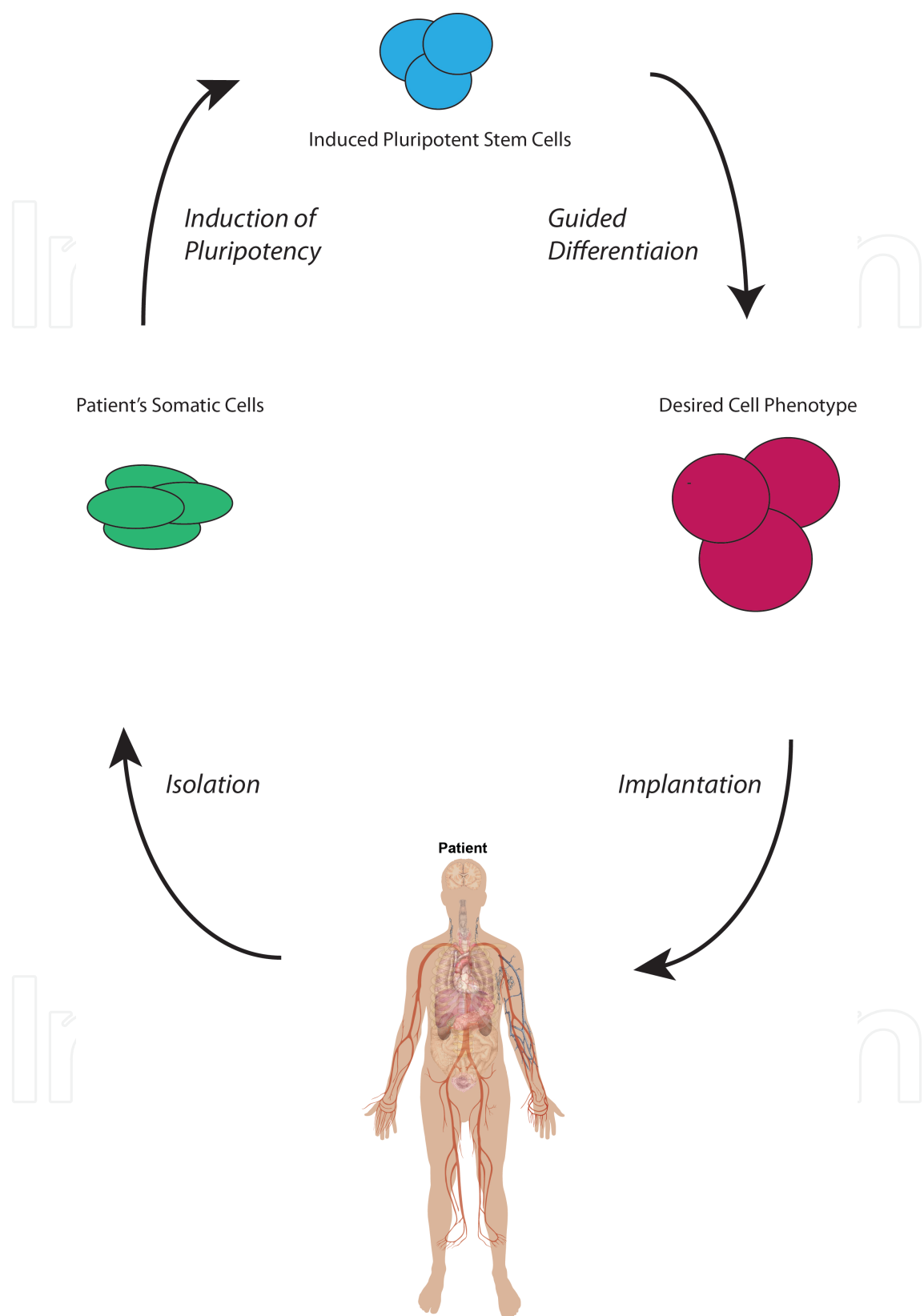
Figure 1. Induced pluripotent treatment pathway: Somatic cells are isolated from the patient, reprogrammed into iPS cells, and then differentiated into the target phenotype for treatment

6.3. Alzheimer's disease

Unlike Parkinson's disease, Alzheimer's disease cannot be traced to the loss of single cell phenotype in a distinct region. In Alzheimer's, damage is diffuse throughout the brain, forming neurofibrillary tangles characterized by high levels of amyloid precursor protein (APP) expression. It has been demonstrated that high levels of APP expression influence differentiation toward the glial phenotype [44], inhibiting direct replacement of neurons through non-terminally differentiated stem cells. As such, Alzheimer's does not lend itself to transplantation therapy as readily as acute neurological injury or Parkinson's. However, there is a potential that transplantation of terminally differentiated neuronal populations derived from iPS cells could have beneficial effects. Transplanted cells may not necessarily replace damaged neurons, but increased neurotrophic factor production from transplanted neuronal populations may have positive effects on patient phenotype.

6.4. Cardiovascular treatments

It has been demonstrated that pluripotent stem cells have the potential to differentiate into cardiomyocyte [45, 46]. Utilizing a number of various culture conditions, including co-culture with stromal cells and cytokine supplementation, differentiation into cardiomyocytes can be made relatively efficient [47]. There is a potential that transplantation of iPS derived cariomyocytes may be able to assist patients who have suffered a myocardial infraction, as has been demonstrated when transplanting other related phenotypes [48]. Studies in a murine model have shown that transplantation of ESC derived cariomyocytes mitigated the functional damage of myocardial infraction [49]. Due to the similarity of iPS cell and ESC phenotypes, there is a potential that similar results would be possible utilizing patient specific iPS cells as the source of cardiomyocytes.

6.5. Hemophilia

Hemophilia is caused by a genetic mutation that reduces the production of coagulant factor VIII or XI depending on the type. Therefore, it's possible that transplantation of iPS derived endothelial cells which express coagulant factors could correct the hemophilia phenotype in patients [50]. In a murine model, transplantation of iPS derived endothelial cells positive for factor VIII expression was able to mitigate the hemophilia A phenotype to a large degree. Endothelial cells were transplanted by injection directly into the liver of hemophiliac mice and functionality was assessed by a tail cutting assay. After treatment, mice with transplanted endothelial cells survived for 3+ months after tail cutting, while control mice died within hours. Factor VIII expression was increased to 8%-12% of normal, indicating that full restoration of factor VIII expression may not be necessary to effectively mitigate the hemophilia phenotype [51]. These findings show promise for the development of cell based therapies to treat hemophilia.

6.6. Blood supply

Blood supply shortages are an ever-present concern in many regions, leading to demand for additional sources of red blood cells (RBCs). iPS cells could theoretically be used to generate RBCs as a supplemental source and it has been demonstrated that iPS cells are capable of direct erythrocytic differentiation [52]. Although the technology exists, the use of iPS cells to generate RBCs may not always been practical due to the cost of iPS generation, culture, and subsequent differentiation. As such, until technologies are developed which allow for industrial scale iPS cell culture and differentiation, the use of iPS cells to augment the blood supply will be fairly limited. In certain circumstances, such as a patient in need of a rare blood type in advance of surgery, generation of iPS derived RBCs may be a viable option for treatment.

7. Research applications

7.1. Disease modeling

Accurate disease modeling is a biotechnological problem of fundamental importance. Most current disease models rely upon murine model organisms, which are capable of providing insight, but are less than ideal due to interspecies differences [53]. iPS technologies could allow for *in vitro* disease modeling, using cultures isolated from those suffering with a given condition. If widely applied, patient specific iPS cultures could potentially be created to analyze the nuances of a disease in a particular patient, determining which course of treatment would be best. Using skin fibroblasts isolated from a patient with spinal muscular atrophy, Ebert et. al. demonstrated that iPS derived motor neurons could be effectively grown in culture and maintained the disease phenotype of the patient [54]. These findings indicate that iPS cells derived from patients with genetic disorders may exhibit the disease phenotype, allowing for their use as a disease model.

Similar isolations have also occurred with Parkinson's patients, in which iPS clones were generated from patients and subsequently differentiated into dopamanergic neurons. However, in the context of Parkinson's disease, the disease phenotype was not as readily presented *in vitro* due to the relative age of the neurons. While cultured neurons have a lifespan in weeks, Parkinson's develops over a period of years due in conjunction with age related factors, possibly requiring a form of artificial stress treatment to accurately reproduce the phenotype *in vitro* [14]. However, early stage metabolic dysfunction has already been identified and corrected *in vitro* using neurons generated from familial Parkinson's patient derived iPS cells, indicating that some early stage phenotypes may be identifiable without full phenotypic replication [55]. Alzheimer's disease, like Parkinson's, is strongly influenced by a number of age related factor which complicate the creation of an accurate model. Recently, Shi et. al. demonstrated one potential approach to this problem by using iPS cells derived from Down syndrome patients. Down syndrome patients overexpress a gene known to encode for amyloid precursor protein (APP), a major component of the Alzheimer's phenotype. Cortical neurons generated from these iPS lines expressed amyloid aggregates and hyperphosphorylated tau protein, both hallmarks of the

Alzheimer's disease phenotype, after months in culture [56]. Utilizing a similar approach, it may be possible to emulate other age related disease phenotypes through variable gene expression, providing a second avenue from which to approach the issue. iPS line have also been derived from Huntington's patients, in which differentiated neurons maintained some portions of the Huntington's phenotype [57, 58]. CDKL5 mutant iPS lines have also been generated from Rett syndrome patients, and may allow for investigation of CDKL5's underlying mechanism within patient cells [59]. Amyotrophic lateral sclerosis (ALS) has also been effectively modeled using an iPS line derived from familial ALS patients [60].

Once generated, these disease models can provide insight into the underlying mechanisms of the disease. *In vitro* research of molecular level cellular mechanisms is much cheaper and more efficient than similar research in mammal models, potentially allowing for increased research throughput. Established *in vitro* models also remove confounding factors related to animal models, potentially making direct identification of mechanisms easier. In the context of phenotypic identification and the discovery of underlying mechanisms, it is important to consider the controls necessary for using these iPS derived disease models. Due to potential phenotypic differences in iPS clones, even from the same isolation, it would be necessary to generate models using multiple iPS lines from each patient in a diverse group. This spread would allow for adequate confirmation that the identified phenotype or mechanism is indeed consistent for all patients with the disease, rather than an artifact of reprogramming or a trait specific to a single individual [53].

While these results in summary are very promising, substantial challenges remain before iPS cell cultures can be used as disease models in every instance. Although diseases with limited temporal dependency, such as spinal muscular atrophy, and clear monogenic origin, such as Huntington's, are replicated relatively easily *in vitro*, there remain unsolved problems in replicating diseases influenced by multiple factors. As demonstrated in attempts to replicate the Parkinson's phenotype *in vitro*, time related factors can also play a large role in disease phenotype, complicating modeling. Other diseases, such as Alzheimer's, may be dependent upon cellular interactions between multiple cell phenotypes in addition to age related factors. The homogenous nature of iPS derived cell cultures complicates accurate replication of these interactions *in vitro*. In some cases, it may be possible to model some of these cell to cell interactions using coculture, as demonstrated in ALS models that incorporate both astrocytes and neurons [61]. Further research is needed to overcome these barriers before iPS cell based disease modeling can be exploited to its full potential.

7.2. Drug discovery

As a corollary to disease modeling, drug discovery is a promising research application for iPS cells. Developing new drugs is exceedingly expensive and many drug candidates are rejected in the final human trial stage due to toxicology concerns [46]. At present, 90% of all drugs candidates that enter clinical trials fail to be approved, leading to a low drug candidate to successful drug ratio [62]. If drugs could be screened for human toxicology earlier in the development cycle, a number of these candidates could be eliminated earlier, allowing for

increased funding to more promising drugs. This redistribution of funding could eventually lead to more drug candidates developed in a more rapid fashion.
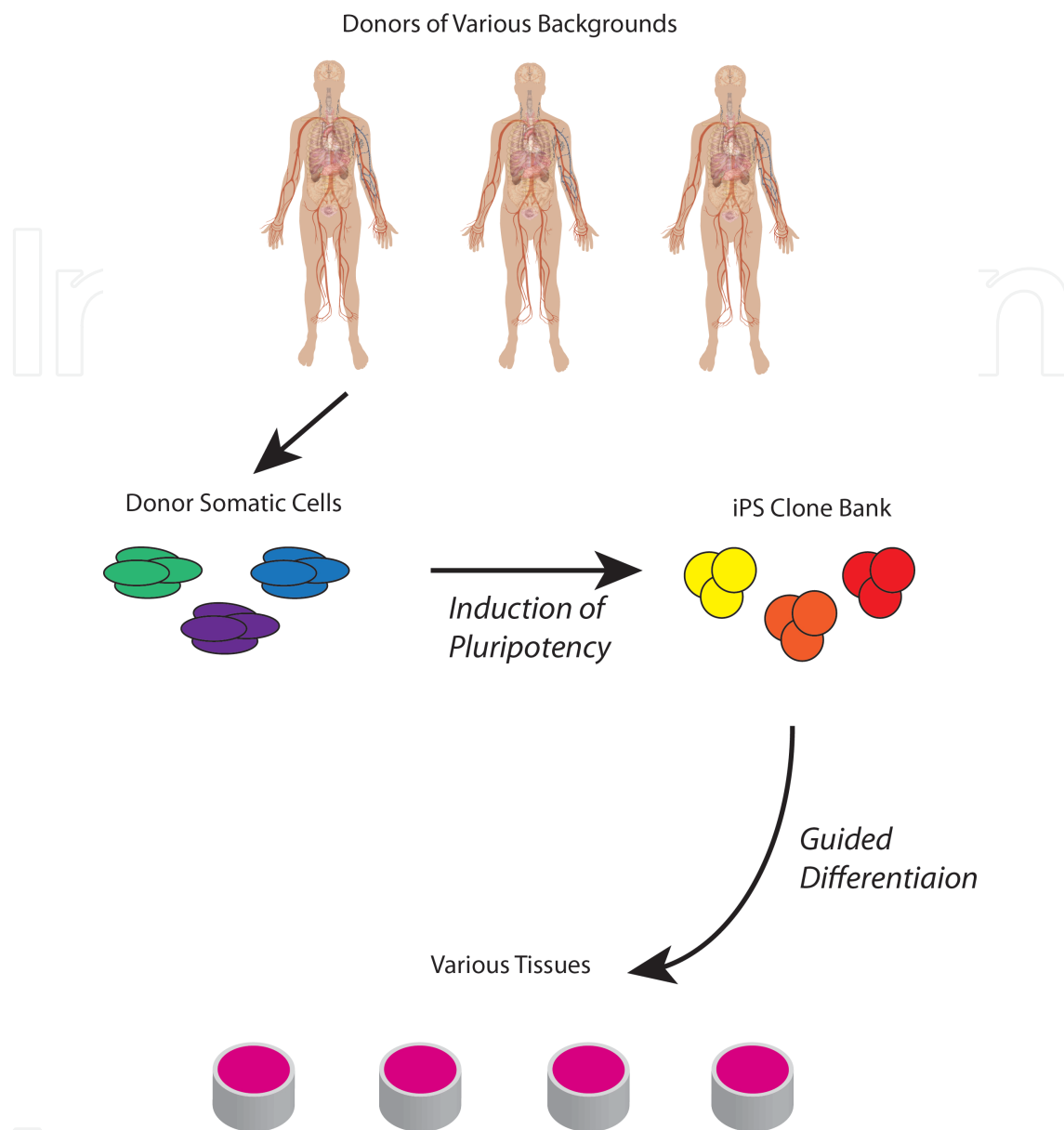
To assess for toxicology, iPS clones could be generated from a broad cross-section of potential patients, representing various patient backgrounds. Due to the immortalized nature of iPS cultures, these cells could be expanded and maintained indefinitely at relatively low expense to drug developers. As a consequence of effective cell storage technologies, an iPS clone bank would only expand overtime, allowing for the aggregation of clones generated during multiple studies. From these clones, tissues could be generated for toxicology testing early in the development cycle, potentially identifying toxic drug candidates before further testing takes place (Figure 2). In this manner, a diverse donor population could effectively provide each type of human tissue with a relatively small amount of tissue collection.

Using iPS disease models as described above, the effectiveness of new drug therapies could also be tested *in vitro*. The overall cost of testing using these *in vitro* models is less than that of animal modeling, and could allow large scale screening of potential drug candidates early in the development cycle. Due to the elimination of certain confounding factors present in animal models, drug testing in iPS derived disease models may also yield unique insights not demonstrated using traditional models. The iPS clone bank described above could be expanded to include similarly diverse clone populations from patients with a specific disease. Similar to its benefits in toxicology testing, an iPS clone bank could allow for testing on a broad cross-section of disease patients at a relatively low cost. Recently, studies have utilized iPS disease models to assess the efficacy of Alzheimer's disease drug candidates *in vitro* [56] and to successfully screen for new drugs to potentially treat ALS [60]. These studies demonstrate the potential for the use of iPS cells in the context of drug development, both to improve the efficiency of existing drug development pipelines and to screen for entirely new compounds in a relatively low cost model.

However, drug discovery and toxicology screening using iPS cells is limited by their ability to accurately replicate *in vivo* conditions. As discussed above, the homogenous nature of iPS cultures neglects many influential factors related to cellular interaction, and the temporally naive nature of iPS cultures neglects many age related factors. As such, further research is necessary before iPS derived tissues are suitable for use in toxicology testing. For drug therapy screening, the current state of disease models as discussed above is a limiting factor. Although not all diseases can be effectively modeled for screening today, some disorders that have well characterized iPS models may benefit from broad drug screening in the near future.

8. Conclusion: Challenges to the road ahead

A number of roadblocks remain before iPS cells are ready for the clinic. At present, there still remains a risk of teratoma formation in the event that a subpopulation of iPS cells is not terminally differentiated prior to transplantation. In the context of a patient-specific autologous treatment using iPS cells, methods must be developed by which iPS cells can be generated in sufficient quantity, reliably, and in a time frame appropriate for the targeted disease.

Donors of Various Backgrounds

Donor Somatic Cells

Induction of Pluripotency

iPS Clone Bank

Guided Differentiaion

Various Tissues

Figure 2. Drug discovery and toxicology workflow. Somatic cells are isolated from a broad cross section of donors, reprogrammed, and differentiated into relevant tissues for toxicology screening and drug testing

Efficiency remains an issue, especially with regards to technologies that do not integrate transgenes into the host genome. To address efficiency concerns, it is possible that application specific optimization of induction technologies could improve the efficacy of current induction technologies.

As disease models, iPS cells are limited by the neglect of several influential factors. Most prominently, the homogenous populations derived from iPS cells inherently neglect interactions between multiple cell phenotypes, and these interactions may be critical to understanding disease mechanisms [6]. iPS cells could potentially be differentiated into various cell types and cocultured to replicate interactions between cell types, but it may be difficult to generate an

accurate interaction model, even with multiple cell types. iPS cell cultures also neglect various age related factors, which may be particularly problematic in modeling certain diseases. These same issues act as barriers to the use of iPS cells for drug discovery and toxicology screening, as both applications rely upon accurate iPS models of *in vivo* cellular activity.

Induced pluripotent stem cell technologies have progressed rapidly in recent years. Various induction methods have eliminated or reduced many of the fundamental issues with iPS cells, opening the door to a variety of possible applications. Though there remain a number of challenges facing the development of iPS cells in the clinic and the laboratory, the potential benefits to regenerative medicine are profound.

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References

[1] Yamanaka, S. and K. Takahashi, *Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors.* Cell, 2006. 126(4): p. 663-676.

[2] Avilion, A.A., et al., *Multipotent cell lineages in early mouse development depend on SOX2 function.* Genes & Development, 2003. 17(1): p. 126-140.

[3] Hong, Y., et al., *Activation of the mouse Oct4 promoter in medaka embryonic stem cells and its use for ablation of spontaneous differentiation.* Mechanisms of Development, 2004. 121(7–8): p. 933-943.

[4] Cartwright, P.P., et al., *LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent mechanism.* Development (Cambridge, England), 2005. 132(5): p. 885-896.

[5] Shi, Y., et al., *A Combined Chemical and Genetic Approach for the Generation of Induced Pluripotent Stem Cells.* Cell Stem Cell, 2008. 2(6): p. 525-528.

[6] Yamanaka, S., *A fresh look at iPS cells.* Cell, 2009. 137(1): p. 13-7.

[7] Junying Yu, et al., *Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells.* Science Express, 2007. 10: p. 1-4.

[8] Liao, J., et al., *Enhanced efficiency of generating induced pluripotent stem (iPS) cells from human somatic cells by a combination of six transcription factors.* Cell Research, 2008. 18: p. 600-603.

[9] Kim, J.B., et al., *Oct4-Induced Pluripotency in Adult Neural Stem Cells.* Cell, 2009. 136(3): p. 411-419.

[10] Shi, Y., et al., *Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds.* Cell Stem Cell, 2008. 3(5): p. 568-74.

[11] Sugaya, K. and A. Alvarez, *Methods and materials for increasing potency of cells* U.P.a.T. Office, Editor 2006: USA.

[12] Alvarez, A., et al., *Nanog overexpression allows human mesenchymal stem cells to differentiate into neural cells——Nanog transdifferentiates mesenchymal stem cells.* Neuroscience and Medicine, 2010: p. 1-13.

[13] Hacein-Bey-Abina, S., et al., *LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1.* Science, 2003. 302(5644): p. 415-9.

[14] Soldner, F., et al., *Parkinson's Disease Patient-Derived Induced Pluripotent Stem Cells Free of Viral Reprogramming Factors.* Cell, 2009. 136(5): p. 964-977.

[15] Okita, K., et al., *Generation of Mouse Induced Pluripotent Stem Cells Without Viral Vectors.* Science (Washington), 2008. 322(5903): p. 949-953.

[16] Yamanaka, S., *Induced Pluripotent Stem Cells: Past, Present, and Future.* Cell Stem Cell, 2012. 10(6): p. 678-684.

[17] Yu, J., et al., *Human induced pluripotent stem cells free of vector and transgene sequences.* Science, 2009. 324(5928): p. 797-801.

[18] Okita, K. and S. Yamanaka, *Induced pluripotent stem cells: opportunities and challenges.* Philos Trans R Soc Lond B Biol Sci, 2011. 366(1575): p. 2198-207.

[19] Armeanu, S., et al., *Cell cycle independent infection and gene transfer by recombinant Sendai viruses.* Journal of Virological Methods, 2003. 108(2): p. 229-233.

[20] Bernloehr, C., et al., *Efficient propagation of single gene deleted recombinant Sendai virus vectors.* Virus Research, 2004. 99(2): p. 193-197.

[21] Tokusumi, T., et al., *Recombinant Sendai viruses expressing different levels of a foreign reporter gene.* Virus Research, 2002. 86(1–2): p. 33-38.

[22] Fusaki, N., et al., *Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome.* Proceedings of the Japan Academy, Series B. Physical and Biological Sciences, 2009. 85B(8): p. 348-362.

[23] Warren, L., et al., *Highly Efficient Reprogramming to Pluripotency and Directed Differentiation of Human Cells with Synthetic Modified mRNA*. Cell Stem Cell, 2010. 7(5): p. 618-630.

[24] Rosa, A. and A.H. Brivanlou, *Synthetic mRNAs: Powerful Tools for Reprogramming and Differentiation of Human Cells*. Cell Stem Cell, 2010. 7(5): p. 549-550.

[25] Zhao, T.T., et al., *Immunogenicity of induced pluripotent stem cells*. Nature, 2011. 474(7350): p. 212-215.

[26] Kim, D., et al., *Generation of Human Induced Pluripotent Stem Cells by Direct Delivery of Reprogramming Proteins*. Cell Stem Cell, 2009. 4(6): p. 472-476.

[27] Shimada, H., et al., *Accelerated generation of human induced pluripotent stem cells with retroviral transduction and chemical inhibitors under physiological hypoxia*. Biochemical and Biophysical Research Communications, 2012. 417(2): p. 659-664.

[28] Yoshida, Y., et al., *Hypoxia enhances the generation of induced pluripotent stem cells*. Cell Stem Cell, 2009. 5(3): p. 237-41.

[29] Lyssiotis, C.A., et al., *Reprogramming of murine fibroblasts to induced pluripotent stem cells with chemical complementation of Klf4*. Proc Natl Acad Sci U S A, 2009. 106(22): p. 8912-7.

[30] Huangfu, D., et al., *Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds*. Nat Biotechnol, 2008. 26(7): p. 795-797.

[31] Hanna, J., et al., *Direct cell reprogramming is a stochastic process amenable to acceleration*. Nature, 2009. 462(7273): p. 595-601.

[32] Loh, Y.H., et al., *The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells*. Nat Genet, 2006. 38(4): p. 431-40.

[33] Papapetrou, E.P., et al., *Stoichiometric and temporal requirements of Oct4, Sox2, Klf4, and c-Myc expression for efficient human iPSC induction and differentiation*. Proceedings of the National Academy of Sciences, USA, 2009. 106(31): p. 12759-12764.

[34] Bar-Nur, O., et al., *Epigenetic Memory and Preferential Lineage-Specific Differentiation in Induced Pluripotent Stem Cells Derived from Human Pancreatic Islet Beta Cells*. Cell Stem Cell, 2011. 9(1): p. 17-23.

[35] Hu, Q.Q., et al., *Memory in induced pluripotent stem cells: reprogrammed human retinal-pigmented epithelial cells show tendency for spontaneous redifferentiation*. Stem cells (Dayton, Ohio), 2010. 28(11): p. 1981-1991.

[36] Kim, K.K., et al., *Epigenetic memory in induced pluripotent stem cells*. Nature, 2010. 467(7313): p. 285-290.

[37] Nori, S., et al., *Transplantation of human iPS cell-derived neurospheres for the treatment of spinal cord injury in NOD-scid mice.* Neuroscience Research, 2010. 68, Supplement 1(0): p. e427.

[38] Uemura, T., et al., *Transplantation of induced pluripotent stem cell-derived neurospheres for peripheral nerve repair.* Biochemical and Biophysical Research Communications, 2012. 419(1): p. 130-135.

[39] Gomi, M., et al., *Functional recovery of the murine brain ischemia model using human induced pluripotent stem cell-derived telencephalic progenitors.* Brain Research, 2012. 1459(0): p. 52-60.

[40] Jensen, M.B., et al., *Survival and Differentiation of Transplanted Neural Stem Cells Derived from Human Induced Pluripotent Stem Cells in A Rat Stroke Model.* Journal of Stroke and Cerebrovascular Diseases, (0).

[41] Cooper, O., et al., *Differentiation of human ES and Parkinson's disease iPS cells into ventral midbrain dopaminergic neurons requires a high activity form of SHH, FGF8a and specific regionalization by retinoic acid.* Molecular and Cellular Neuroscience, 2010. 45(3): p. 258-266.

[42] Swistowski, A., et al., *Efficient generation of functional dopaminergic neurons from human induced pluripotent stem cells under defined conditions.* Stem Cells, 2010. 28(10): p. 1893-904.

[43] Wernig, M., et al., *Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease.* Proceedings of the National Academy of Sciences, USA, 2008. 105(15): p. 5856-5861.

[44] Kiminobu Sugaya, S.M., *How to approach alzheimer's disease with stem cell technologies.* Journal of Alzheimer's Disease, 2008. 15: p. 241–254.

[45] Taha, M.F. and V. Hedayati, *Isolation, identification and multipotential differentiation of mouse adipose tissue-derived stem cells.* Tissue Cell, 2010. 42(4): p. 211-6.

[46] Nsair, A. and W.R. MacLellan, *Induced pluripotent stem cells for regenerative cardiovascular therapies and biomedical discovery.* Advanced Drug Delivery Reviews, 2011. 63(4–5): p. 324-330.

[47] Yoshida, Y. and S. Yamanaka, *iPS cells: A source of cardiac regeneration.* Journal of Molecular and Cellular Cardiology, 2011. 50(2): p. 327-332.

[48] Galli, D., et al., *Mesoangioblasts, vessel-associated multipotent stem cells, repair the infarcted heart by multiple cellular mechanisms: a comparison with bone marrow progenitors, fibroblasts, and endothelial cells.* Arterioscler Thromb Vasc Biol, 2005. 25(4): p. 692-7.

[49] Christoforou, N.N., et al., *Implantation of mouse embryonic stem cell-derived cardiac progenitor cells preserves function of infarcted murine hearts.* PLoS ONE, 2010. 5(7): p. e11536-e11536.

[50] Liras, A., *Induced human pluripotent stem cells and advanced therapies: Future perspectives for the treatment of haemophilia?* Thrombosis Research, 2011. 128(1): p. 8-13.

[51] Xu, D.D., et al., *Phenotypic correction of murine hemophilia A using an iPS cell-based therapy.* Proceedings of the National Academy of Sciences of the United States of America, 2009. 106(3): p. 808-813.

[52] Peyrard, T., et al., *Banking of Pluripotent Adult Stem Cells as an Unlimited Source for Red Blood Cell Production: Potential Applications for Alloimmunized Patients and Rare Blood Challenges.* Transfusion Medicine Reviews, 2011. 25(3): p. 206-216.

[53] Saha, K. and R. Jaenisch, *Technical Challenges in Using Human Induced Pluripotent Stem Cells to Model Disease.* Cell Stem Cell, 2009. 5(6): p. 584-595.

[54] Ebert, A.D., et al., *Induced pluripotent stem cells from a spinal muscular atrophy patient.* Nature, 2009. 457(7227): p. 277-280.

[55] Cooper, O., et al., *Pharmacological Rescue of Mitochondrial Deficits in iPSC-Derived Neural Cells from Patients with Familial Parkinson's Disease.* Science Translational Medicine, 2012. 4(141): p. Article.

[56] Shi, Y., et al., *A Human Stem Cell Model of Early Alzheimer's Disease Pathology in Down Syndrome.* Science Translational Medicine, 2012. 4(124): p. 124ra29.

[57] Castiglioni, V., et al., *Induced pluripotent stem cell lines from Huntington's disease mice undergo neuronal differentiation while showing alterations in the lysosomal pathway.* Neurobiology of Disease, 2012. 46(1): p. 30-40.

[58] Camnasio, S., et al., *The first reported generation of several induced pluripotent stem cell lines from homozygous and heterozygous Huntington's disease patients demonstrates mutation related enhanced lysosomal activity.* Neurobiology of Disease, 2012. 46(1): p. 41-51.

[59] Amenduni, M., et al., *iPS cells to model CDKL5-related disorders.* Eur J Hum Genet, 2011. 19(12): p. 1246-55.

[60] Egawa, N., et al., *Drug Screening for ALS Using Patient-Specific Induced Pluripotent Stem Cells.* Science Translational Medicine, 2012. 4(145): p. 145ra104.

[61] Di Giorgio, F.P., et al., *Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model.* Nat Neurosci, 2007. 10(5): p. 608-614.

[62] Rubin, L.L., *Stem Cells and Drug Discovery: The Beginning of a New Era?* Cell, 2008. 132(4): p. 549-552.