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Induced pluripotent stem cells (iPSCs): uprising in favor of Medical Biotechnology

Manas Kanti Saha[1], Abdullah-Al-Emran[2*]

[1]*Department of Biotechnology, Bangladesh Agricultural University, Mymensingh, Bangladesh*

[2]*Department of Biotechnology and Genetic Engineering, Mawlana Bhashani Science and Technology University, Santosh,Tangail, Bangladesh*

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Abstract

Induced Pluripotent Stem Cells (iPSCs) are stem cells that are reprogrammed genetically from somatic cells to exhibit pluripotent characteristics. The generation of iPSCs from somatic cells demonstrated that adult mammalian cells can be reprogrammed to a pluripotent state by the enforced expression of a few embryonic transcription factors. Pluripotent stem cells possess the unique property of differentiating into all other cell types. The discovery iPSCs in 2006 has led new avenues and dimension in clinical medicine. In addition, iPSC technology has provided researchers with a unique tool to derive disease-specific stem cells for the study and possible treatment of degenerative disorders with autologous cells. These models can also be used to study the safety and efficacy of known drugs or potential drug candidates for a particular diseased condition, limiting the need for animal studies and considerably reducing the time and money required to develop new drugs. Recently, functional neurons, cardiomyocytes, pancreatic islet cells, hepatocytes and retinal cells have been derived from human iPSCs, thus re-confirming the pluripotency and differentiation capacity of these cells. These findings further open up the possibility of using iPSCs in cell replacement therapy for various degenerative disorders. iPSC are also uprising to develop personalized treatment, vaccination system, toxicological and pharmacological screening those are very important sector related to Medical Biotechnology.

* **Corresponding Author:** Abdullah Al Emran, ✉ emrangeb@gmail.com

Introduction

Simply, Medical Biotechnology is such kind of technology that has revolutionized Medical Science. It is a rapidly emerging technology for improvement of health and nutrition (Biancotti *et al.,* 2010). It uses biological agents, including GMOs, to get medical products and services. Important areas of medical biotechnology include pharmaceutical products, vaccines, diagnostic techniques such as PCR and monoclonal antibodies, transgenic animals, microarray, nano-medicine, bioinformatics, pluripotent cells for development of any type of adult tissue, antisense technology and gene therapy (Chang *et al.,* 2010). Among all of them development of pluripotent cells and their application in drug development, disease remodeling, cell therapy etc. (DeKelver *et al.,* 2010) is the most recent concern for researchers. Embryonic stem cells are unspecialized or undifferentiated cells (Ferrante *et al.,* 2009) that can divide indefinitely in culture and can develop into specialized or differentiated cells (Guo *et al.,* 2009). First few days later of fertilization of an ovum, stem cells convert into totipotent, that is, they have the potential to become a complete organism, such as a human being. Generally four days later, the totipotent cells form blastocyst becomes a little more specialized. pluripotent cells (Hagerman *et al.,* 2002), that have a more restricted potential, make up the outer layer of the blastocyst and give rise to the placenta and other tissues required to sustain fetal development. A second type of pluripotent cells form the so-called inner cell mass of the blastocyst and will give rise to most of the tissues in the body (Hussein *et al.,* 2011). These embryonic pluripotent cells are the stem cells of interest to science and medicine. Actually pluripotent cells cannot generate a complete organism, but in normal development they do produce specialized, or multipotent, stem cells in the fetus or adult animal which produce the differentiated cells that make up the different components of the body (Jackson *et al.,* 2001). So we can say, pluripotency is the capability of a cell to give rise to all supplementary cell types. Actually blastocyst is the source of such kind of cells. It is essential to implant these cells in embryo for their persistence (Maitra *et al.,* 2005). It is also possible to develop disease-specific iPSCs which are most likely to revolutionize research in respect to the pathophysiology of most debilitating diseases, as these can be mimicked *ex vivo* in the laboratory. These models can also be used to study the safety and efficacy of known drugs or potential drug candidates for a particular diseased condition, limiting the need for animal studies and considerably reducing the time and money required to develop new drugs. (Yamanaka *et al.,* 2009) World famous scientists have been involved in this sector for last decades. Human iPS cell derivation previously required vectors that integrate into the genome, which can create mutations and limit the utility of the cells in both research and clinical applications (Hall *et al.,* 2009). Now it is possible the derivation of human iPS cells with the use of non integrating episomal vectors. Ethical and technical concerns are important obstacle to generate pluripotent cells (Pick *et al.,* 2009). This objective gained even more importance when ethical and other technical concerns, such as tumor formation and immune rejection, severely restricted research with human embryonic stem cells (hESCs) (Aoki *et al.*,2010). Previous attempts at somatic cell nuclear transfer (cloning) and fusion of somatic cells with embryonic cells was marred by various ethical and methodological complications) (Anokye *et al.*, 2011), which precluded their use as a routine research tool. However, it is clear that success in reprogramming adult cell lines could lead to cell lines which could emerge as excellent research tools to understand diseases and to test potential drug treatments (Aoi *et al.,* 2008). Also, the possibility of using cells to repair damaged organs would be available (Virginia, 2011) and the cell lines would be immune to rejection as they would be derived from the patient him/herself (Das *et al.,* 2010). In this work historical background, the development of iPSCs by different methods and their biological characteristics, their prospective applications in medical biotechnology, some practical challenges as well as future perspective related to this technology and how they can be averted for the betterment of human life were reviewed. The overall work plan is given in Fig. 1.

Historical background

Discovery of induced pluripotent stem cell was not a single day task. Many researchers were concerned to establish effectively such class of cells. At first they had to demonstrate that vastly differentiated cells preserve the same genetic information as early embryonic cells and then they developed a number of techniques to derive culture as well as study pluripotent cell lines. Actually the demonstration of John B. Gurdon , in 1962, about the capability of generating a fully functional tadpole from a nucleus of a differentiated frog intestine epithelial cell, was the first scientific achievement in this field. This discovery devastated the belief that cellular differentiation could only be a unidirectional procedure. After a long time study researchers observed that in fact transcription factors are key determinants of cell fate and the expression of that kind of factor can switch one mature cell type into another. At last in 2012, Dr. John B Gurdon and Dr. Shinya Yamanaka awarded Nobel Prize in Physiology for the discovery that "Mature, differentiated cells can be reprogrammed to a pluripotent stem cell state". The important research works related to development of induces pluripotent cells are listed at Table1.

Table 1. A list of iPSCs related research work.

Year of Discovery	Research Work	Reference
1950	Establishment of the technique of Stem cell nuclear transplantation.	Briggs and King, 1952
1962	Differentiated amphibian cells indeed retain the genetic information to support the generation of cloned frogs.	Gurdon, 1962
1972	Establishment of immortal pluripotent cell lines from teratocarcinomas, tumors of germ cell origin.	Brinster, 1974
1976	Hybrid cells acquired biochemical and developmental properties of ECCs and extinguished features of the somatic fusion partner.	Miller,1976; Ruddle ,1977
1980	Derivation of embryonic stem cells (ESCs) from the inner cell mass (ICM) of mouse blastocysts.	Evans and Kaufman, 1981; Martin 1981
1986	Formation of myofibers in fibroblast cell lines transduced with retroviral vectors expressing the skeletal muscle factor MyoD.	Davis *et al.* 1987
1990	Capability of producing entirely ESC-derived animals after injection into tetraploid blastocysts.	Nagy *et al.* 1990
2006	Induction of pluripotent stem cells from embryoinic and adult fibroblast cultures by defined factors.	Takahashi and yamanak, 2006
2007	Generation of germline- competent induced pluripotent cells.	Okita *et al.,* 2007
2008	Generation of mouse induced pluripotent cells without viral infection.	Stadtfeld *et al.,* 2008
2009	Generation of induced pluripotent cells from patients with type 1diabetes	Maehr *et al.,* 2009
2009	Modeling pathogenesis and treatment of familial dysautomnia using patient specific iPSCs.	Lee *et al.*, 2009
2011	Somatic coding mutations in human induced pluripotent stem cells.	Gore A *et al.*, 2011
2012	"Mature, differentiated cells can be reprogrammed to a pluripotent stem cell state.	Nobel Prize in Physiology, 2012

Induced pluripotent stem cells (iPSCs)

It is such kind of pluripotent stem cell that is artificially derived from a somatic cell via inducing an expression of specific gene (Araki *et al.*, 2010). Actually it is similar to embryonic (EM) cell but it has the capability to generate several cells in the body (Takahashi and Yamanaka, 2006). To develop iPCs, first of all embryonic stem cells are isolated from the inner cell mass (Bao *et al.,* 2009) of any model organism including mouse, monkey, pig, marmoset and human blastocysts. Then those cells may be prolonged in culture while retaining the capacity to construct all cells in the body (Brambrink *et al.*, 2008). The only one distinguishing is that they retain

epigenetic memory from the source tissue (Draper *et al*, 2006). The existing obstacle with iPSC lies is their low efficiency of derivation (Feldman *et al*, 2006) and the heterogeneity of the obtained colonies (Ghosh *et al.*, 2010). The morphological appearance, proliferation rate, the reactivation of endogenous pluripotency genes followed by silencing of transgenes used for reprogramming (Hajkova *et al.*, 2008), and the ability to form teratomas are a number of of the fundamental criteria for the assortment of a "excellent quality" iPSC (Han *et al.,* 2008). Direct reprogramming of human somatic cells into pluripotency is very essential to generate patient-specific iPSCs for disease modeling (Kim *et al.,* 2009) and cellular replacement therapies (Marion *et al.,* 2009). However it is difficult due to efficiency and safety issues associated with generation of human iPSCs (Stadtfeld *et al.*, 2010). To date, not all of the cell type are not effective for human iPSCs development. Fibroblasts, keratinocytes and neural cell are the best choice due to their wide availability, easy isolation and stable genetic characteristics (Eminli *et al.* 2008). In addition to, iPSCs have also been derived from other somatic cell populations including stomach, liver cells (Aoi *et al.* 2008), melanocytes (Utikal *et al.* 2009), as well as from genetically labeled pancreatic b cells (Lin *et al.*, 2009) and terminally differentiated lymphocytes (Oswald *et al.*, 2000).

Major genes and transcription factor for stimulation iPSC

In August 2006, Takahashi and Yamanaka investigated that *Oct-3/4, SOX2, c-Myc, and Klf4* genes are essential for the production of iPSC (Takahashi and Yamanaka, 2006). These genes had been identified as particularly important in embryonic stem cells (ESCs) (Santiago *et al.*, 2008), and used retroviruses to transduce mouse fibroblasts with a selection of those genes (Yu *et al.*, 2009). Since two of the four genes used (namely, *c-Myc* and *KLF4*) are oncogenic (Zalfa *et al.*, 2003) , and 20% of the chimeric mice developed cance (Zhou *et al.,* 2004), then another research groups from Harvard, MIT, and the University of California, Los Angeles, showed successful reprogramming of mouse fibroblasts into iPS cells and able to produce viable chimera by using *Nanog* and *LIN28* which are important genes in ESCs involved DNA methylation patterns (Tokumoto *et al.,* 2010). The findings of them indicated that Nanog is a major determinant (Urbach *et al.,* 2010) of cellular pluripotency. In another study, Takahashi and Yamanaka again that one can create iPSCs yet without c-Myc (Shi *et al.,* 2008). The process takes longer and is not as efficient, but the resulting chimeras didn't develop cancer (Winkler *et al.,* 2010). They also reported that *Nanog and LIN28* was unnecessary for induction to generate iPS cells (Seandel *et al.,* 2007). Embryonic cell specific microRNA molecules including *miR-291, miR-294 and miR-295* increase the effectiveness of induced pluripotency by acting downstream of c-Myc (Tahiliani *et al.,* 2009). On the other hand, transcription factors help to establish and maintain cellular individuality during development by driving the expression of cell type-specific genes while suppressing lineage inappropriate genes (Pfannkuche *et al.,* 2010). The role of transcription factors was first demonstrated by the formation of myofibers in fibroblast cell lines transduced with retroviral vectors expressing the skeletal muscle factor MyoD (Davis *et al.,* 1987). Subsequently, Graf and colleague (Xie *et al.,* 2004; Laiosa *et al.,* 2006) investigated that primary B and T cells could be converted efficiently into functional macrophages upon over expression of the myeloid transcription factor C/EBPa (Zhao *et al.,* 2004). More recently, researchers have identified sets of transcription factors that induce the conversion of pancreatic acinar cells into insulin-producing b cells by overexpressing the pancreatic factors MafA, Pdx1, and Ngn3 (Zhou *et al.* 2008); the conversion of fibroblasts into neurons by the activation of the neural factors Ascl1, Brn2, and Myt1l (Vierbuchen *et al.* 2010); and the conversion of fibroblasts into cardiomyocytes by the cardiac factors Gata4, Mef2c, and Tbx5 (Ieda *et al.,* 2010).

Different approaches of integration

A number of different approaches have been established to transfer reprogramming factors into somatic cells (Zou *et al.,* 2009), which have an effect

on the efficiency of reprogramming and the superiority of resultant iPSCs (Vaziri *et al.,* 2010). First of all *virus mediated integration* uses adenovirus to transport the requisite four transcription factors into the DNA of skin and liver cells of mice (Tokuzawa *et al.,* 2003). The adenovirus is unique from other vectors like viruses and retroviruses because it does not incorporate any of its own genes into the targeted host and avoid the potential for insertional mutagenesis (Bruck *et al.,* 2007). Another popular approach is *plasmid, minicircle and transposon mediated integration* (Okita *et al.,* 2008). Two plasmid vectors are used successfully to reprogram mouse cells (Sutcliff *et al.,* 1992). The first plasmid expressed c-Myc, while the second expressed the other three factors (Oct4, Klf4, and Sox2) (Wilmut *et al.,* 1997). But it has risk of insertional mutagenesis Thomson *et al.*, 1998). *Transposon system* is better than retroviral approach due to its higher effeiciency (Bilic *et al.*, 2012). *Protein mediated approach* is cumbersome and requires recombinant protein expression and purification expertise, and reprograms albeit at very low frequencies (Song *et al.,* 2010). It can avoid DNA integration concerns as well as providing better control over the concentration, timing and sequence of transcription factor stimulation. Another research group demonstrated that polyarginine peptide conjugation can deliver recombinant protein reprogramming factor (RF) cargoes into cells and reprogramme somatic cells into iPSCs (Zhou *et al.*, 2009). However, the protein-based approach requires a significant amount of protein for the reprogramming process (Mali *et al.,* 2010). IVT RNA transduction uses single-stranded RNA biotypes that trigger innate antiviral defense pathways such as interferon and NF-κB-dependent pathways (Varas *et al.,* 2009). In vitro transcribed RNA, containing stabilizing modifications such as 5-methylguanosine capping (Aasen *et al.,* 2008). It is more efficient than viral transduction (Xu *et al.*, 2009) and has the extra advantage of not altering the somatic genome (Polo *et al.,* 2010). In adding up *small molecule mediated approach* is used to replace genes with small molecules to assist in reprogramming (Silva *et al.,* 2006). It has moderate efficiency. Vector related approaches have some obstacles (Lengner *et al.,* 2010). Vectors can produce insertional mutations that may interfere with the normal function of iPS cell derivatives, and residual transgene expression can influence differentiation into specific lineages (Niclis *et al.,* 2009) or even result in tumorigenesis (Buecker *et al.,* 2010) . To overcome those limitations, *Vector free integration* has arisen as the latest approach (Perrier *et al.,* 2004). Basically there are two approaches to remove trasgenes from iPSC cells such as Cre/LoxP recombination that is involved to excise integrated transgene (Giorgetti *et al.,* 2009) as well as PiggyBac trasposons that has not yet been reported (Ohi *et al.,* 2011). Although removing of multiple transposons is labor intensive (Inoue *et al.,* 2011).

Development of iPSC

We know that dedifferentiation is the reversion process of differentiation. Differentiation is such kind of process by which a single stem cell is differentiated into somatic cell (Sipione *et al.,* 2002). On the other hand somatic cell is converted into stem cell due to dedifferentiation (Maherali *et al.,* 2008). Basically there are several ways to reprogram somatic cells into stem cells. First, it can be done by transplantation of nuclei taken from somatic cells into a fertilized egg or oocyt from which the nucleus is removed prior (Eggan *et al.,* 2001). Second, modification of somatic cells, inducing its transformation into a stem cell using the genetic material encoding reprogramming protein factors, recombinant proteins, microRNA, and low-molecular biologically active substances (Irwin *et al.,* 2001). Third, Fusion of somatic cells with pluripotent stem cells (Ogonuki *et al.,* 2002). Actually the fundamental biology of iPSC development is theoretically uncomplicated and efficient. However, the authentic methodology consists of a number of steps, each of which is technically challenging, in due course making it tiresome and requiring sophisticated scientific skills as well as laboratory facilities (Huangfu *et al.,* 2008) After a long research now only four essential transcription factors are used for reprogramming in transforming different cell types(Kunisato *et al.,* 2009).. Although c-Myc (a

proto-oncogene) was found to induce tumors in mice and hence was excluded from the reprogramming basket, albeit at the cost of the efficiency of the process (Cowan *et al.*, 2005). This subtle modification has also rendered the process more time consuming, since c-Myc plays a significant role in augmenting the rate of dissemination of the somatic cells, thereby making them more amenable to reprogramming. The transmission of these transcription factors was a carried out using nucleic acid-based delivery of the programming factors. Due to some limitations of vector mediated methods, non-integrating methods are becoming popular day by day (Lei *et al.*, 1996). Generation of iPSCs free of vector and transgene sequences using non integrating episomal vectors was shown (Yu *et al.*, 2009). (Park *et al.*, 2008) Actually, viral and plasmid DNA incorporation into chromosomes can lead to the disruption of gene transcription and even malignant transformation (Kawamura *et al.*, 2009). Reprogramming should be attempted with transient gene expression to generate iPSCs for human therapy. Although adenoviral vectors have been used to construct mouse iPSCs without viral integration, followed by successful creation of human iPSCs from embryonic fibroblasts (Kleinsmith *et al.*, 2008), using adenoviral vectors expressing c-Myc, Klf4, Oct4 and Sox2 (Zhou *et al.*, 2009). To date, iPSCs have been successfully generated using lentiviruses, retroviruses, adenoviruses, plasmids, transposons and recombinant proteins (Kim *et al.*, 2010). A schematic diagram for development of iPSCs is shown in Figure 2 (Modified from Virginia *et al.*, 2011).

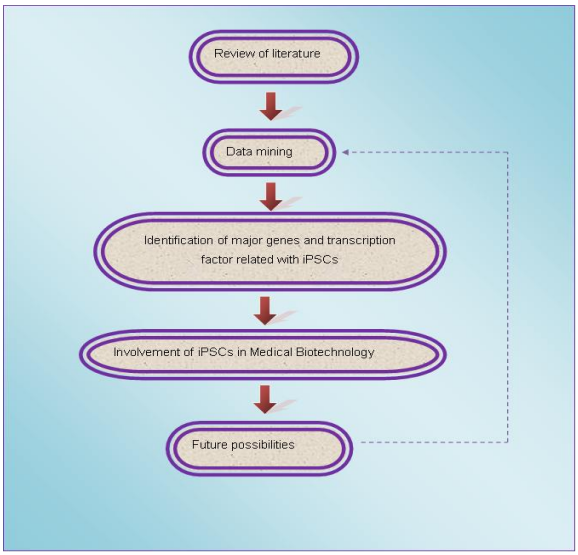

Fig. 1. Overview of research work.

iPSCs and Medical Biotechnology Treatment of genetic disorder

It was impossible to treat the patients of genetic disorder. The study of treatment of genetic disorder is limited by the accessibility of the affected tissues (Verlinsky *et al.*, 2005), as well as the inability to grow the relevant cell types in culture for extended periods of time (Liao *et al.*, 2009). But the iPSC technology opens a new era for such kind of treatment.

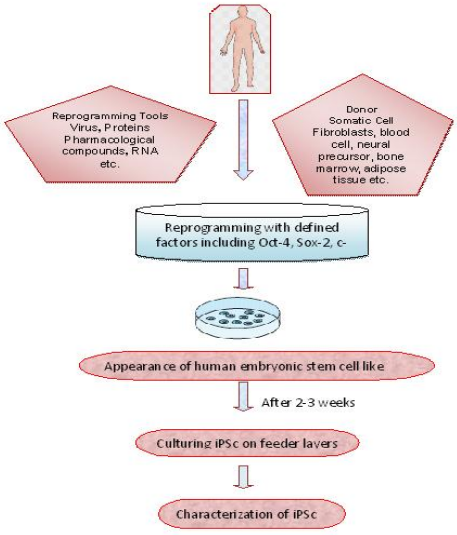

Fig. 2. Development of iPSc (Modified from Virginia *et al.*, 2011).

Type 1 diabetes (T1D)

Actually it is the result of an autoimmune disease caused by destruction of pancreatic β cells (Lin *et al.,* 2001). To date the molecular and cellular reasons behind this disease remain unclear top the researchers (Zhang *et al.,* 2009). Nevertheless pluripotent stem cells generated from patients with T1D would be very useful for understanding the disease modeling (Maehr *et al.,* 2009). Maehr *et al.* published strong report in 2009 showing generation of iPSCs from patients with T1D (Maehr *et al.*, 2009). Adult fibroblasts from T1D patients were efficiently reprogrammed to iPSCs(Koch *et al.,* 2009) using three transcription factors, OCT4, SOX2 and KLF4 (Deng *et al.,* 2009). Such kind of disease-specific stem cells recommend an unprecedented prospect to run through both habitual and pathological human tissue formation *in vitro* (Nichols *et al.,* 2009), in this manner enabling disease exploration and drug development (Tateishi *et al.,* 2008).

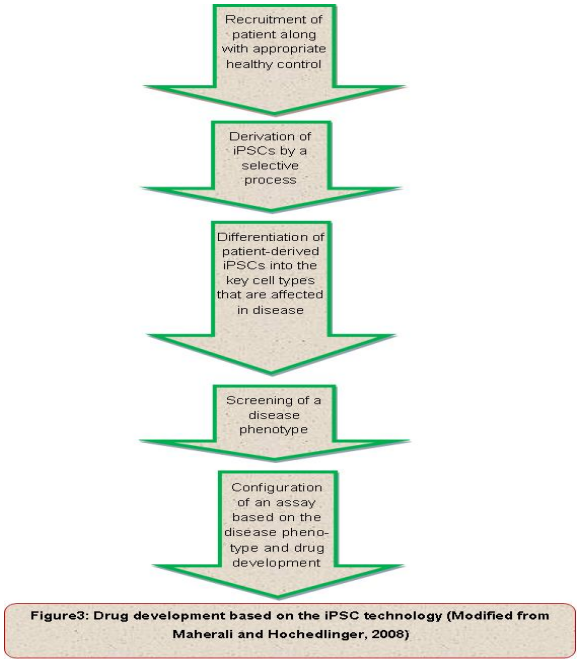

Fig. 3. Drug development based on the iPSC technology (Modified from Maherali and Hochedlinger, 2008).

Spinal muscular atrophy (SMA)

SMA is one kind of autosomal recessive childhood disease that caused by a decline in levels of the survival of motor neuron (SMN) protein due to mutations in the SMN1 gene (Lefebvre *et al.,* 1995) as well as it is the most common cause of death by a heritable disease in infants (Coovert *et al.,* 1997). A group of scientists created two iPSC lines one from a patient with SMA and the other from an unaffected relative and differentiated them into motor neurons (Chambers *et al.,* 2004). They used two compounds, valproic acid and tobramy-cin that actually played role to increase the number of SMN-rich structures (called gems) in the patient derived iPSCs (Avila *et al.,* 2007). In this study motor neuron numbers were reduced, particularly in the cells consequent from patients with SMA, signifying for the first time that the process of reprogramming and directed differ-entiation faithfully captured and recapitulated the disease phenotype (Marica *et al.*, 2011). These iPS cells initially generated a similar number of motor neurons as their control cell counterparts, but over time cell body size was reduced and they underwent substantial degeneration (Okita *et al.*, 2007). It should be mentioned that SMA has four subtypes designated as type-1, 2, 3, 4 that are classified by disease severity (Mattis *et al.,* 2009) and age of onset, with type 1 being the most severe (Zhou *et al.,* 2008) and type 4 being the least severe (Desponts *et al.,* 2010).

Rett Syndrome

Rett syndrome is an X-linked disorder that is a part of the larger group of autism spectrum disorders as well as caused by mutations in methyl-CpG-binding protein 2 *(MECP2)* (Okada *et al.*, 2008) that is involved in DNA methylation (Amir *et al.,* 2000). Such kind of protein actually regulates an array of different gene. It was investigated that most patients are female, as male fetuses or neonates with Rett's syndrome die (Ko *et al.*, 2009), respectively, before or soon after birth due to the pattern of X-chromosome inactivation (Marchetto *et al.,* 2010). A research group generated iPSCs derived from healthy controls and patients with Rett syndrome were differentiated into glutamatergic and GABA (γ-aminobutyric acid)-ergic neurons (Onorati *et al.,* 2010). That group observed no changes in neurogenesis (Maekawa *et al.*, 2011), they were able to measure a substantial reduction in synapse number as well as a reduction in

the number of spines the small protrusions in neuronal processes where glutamatergic synapses are formed while a concentrated number of spines have formerly been experiential in the post-mortem brains of patients with Rett syndrome (Marica *et al.*, 2011).

Parkinson's disease

It is another kind of genetic (neurodegenerative) disorder that is caused by the progressive loss of midbrain dopaminergic neurons (Wichterle *et al.,* 2002). Like all of the genetic disorder it was impossible to cure from this disease. But recently several researches are going on by the use of iPSC technology (Barber*i et al.,* 2003). Many genes have been directly associated with Parkinson's disease (*PARK2*, *SNCA*, *UCHL1*, *LRRK2*, *PARK7*, *PINK1*, *GBA*, and *SNCAIP*) while more than 85% of Parkinson's disease cases give the impression to be irregular (Yamashita *et al.,* 2006). Seibler *et al.* derived dopaminergic neurons from patients with mutations in the gene encoding PTEN-induced putative kinase 1(Cooper *et al.,* 2008), a surface mitochondrial membrane protein that is whispered to normalize the mitochondrial translocation of the E3 ubiquitin protein ligase parkin that is also associated with familial Parkinson's disease.Generation of iPS cells from patients with Parkinson's disease has been described in three reports (Dawson *et al.,* 2007). It was shown that iPS cells with a mutation in *PINK1* were differentiated into dopaminergic neurons. Amusingly these phenotypes were all inverted after in excess of expression of wildtype *PINK1* (Soldner *et al.,* 2009).

Huntington's disease

It is a common autosomal dominant neurodegenerative disease which is caused by expanded CAG repeats in exon 1 of *Huntingtin (HTT protein)* (Roses *et al.,* 1994). It is a disorder related to ageing (Cepeda *et al.,* 2003). Such kind of expantion is caused by mutation on histone deacetylase (had-3) that generates Huntingtin polyglutamine toxicity which is actually responsible for neurodegeneration (Varani *et al.,* 2003). First of all Park *et al.,* generated iPS cells from a patient with Huntington's disease displaying 72 CAG repeats (Seo *et al.,* 2004). These cells have been used to produce striatal neurons subject to cellular damage characteristic of the disease (Xie *et al.,* 2004), such as mutant huntingtin aggregation (Yan *et al.,* 2005) and decreasing concentrations (Shelbourne *et al.,* 2007) of glutamate transporters (Trettel *et al.,* 2008 ; Miller *et al.,* 2008).

Fragile X syndrome

It is an X-linked dominant disorder (Hinton *et al.,* 1991) that is caused by expansion of a tri nucleotide sequence (Rousseau *et al.,* 1992) repeat of more than 200 CGG repeats in the 5´ UTR that silences *FMR1* (Siomi *et al.,* 1993) and ultimately leads to developmental changes within the cerebral cortex (Verkerk *et al.,* 1991) as a result causes mental retardation (Churchill *et al.,* 2004). The developed iPS cells continued to silence the expanded copy of *FMR1* (Crawford *et al.,* 2001), which would not be expected if the cells were pushed back to an embryonic state where the gene would normally be expressed (Castren *et al.,* 2005). However, the FXS iPS cells still represent an exciting model to further analysis of this disorder (Bechara *et al.,* 2009).

Hutchinson-Gilford progeria syndrome (HGPS)

It is an autosomal dominant disorder that is a result of a mutation in the lamin A (*LMNA*) gene(Wang *et al.*, 2006) which leads to a truncated and farnesylated form of LMNA called progerin (Wilson *et al.*, 2009). Patients carrying mutations in *LMNA* show signs of early ageing (Winkler *et al.*, 2010) and often die in their early teens as a result of myocardial infarction or stroke. Several tissues such as mesenchymal lineage cell, vascular smooth muscle cells (VSMCs) are ravaged by such kind of disorder (Martinez *et al.*, 2010). Zhang *et al.* developed iPSCs from patients with HGPS carrying different mutations in *LMNA* (Zhang *et al.,* 2009) while iPSCs developed from their parents were used as controls as well as differentiated (Wu *et al.*, 2010) these cells into five lineages: fibroblasts, endothelial cells, neural progenitor cells, VSMCs and mesenchymal stem cells (Marcia *et al.*, 2011).

Down's syndrome

It is one kind of disorder that is caused by trisomy of chromosome 21(Bahn *et al.,* 2002). iPS model of a Down's syndrome was generated (Osafune *et al.,* 2008) and did enable continuous replay of cortical development (Osakada *et al.,* 2008). The creation of iPS cell lines to enable investigation of similar defects, such as trisomy in other chromosomes, would also be of interest (Bhattacharyya *et al.,* 2009; Matsui *et al.,* 2010).

Long QT syndrome (LQTS)

It is an inherited congenital disorder (Matsui *et al.*, 1992) that is characterized by delayed repolarization of the cardiomyocyte action potential and a prolonged QT interval (A measure of the time between the start of the Q wave and the end of the T wave in the electrical cycle of the heart) in electrocardiograms (Wakayama *et al.*, 2001). Actually the genetic mutations associated with LQTS has hindered attempts to develop protective drugs for this condition (Wakayama *et al.*, 2006), as well as attempts to screen preclinical drug candidates to eliminate those drugs that promote arrhythmia (Kim *et al.*, 2010). Some renowned scientists were triumphant to derive iPSCs from patients with LQTS as well as differentiated them into cardiomyocytes and documented phenotypes that are pinpointing of LQTS (Page *et al.*, 2009). Among themat first Moretti *et al.* developed iPSCs from family members of affected by type 1 LQTS who was actually a carrier of the corresponding mutation in the gene encoding potassium voltage-gated channel subfamily KQT member 1(Pasi *et al.*, 2011) . Cardiomyocytes derived from these iPSCs exhibited prolonged action potentials and defective potassium channel properties (Marica *et al.*, 2011).

Amyotrophic lateral sclerosis (ALS)

It is one kind of genetic disease caused by the death of upper and lower motor neurons, which leads to paralysis and subsequent atrophy of the muscles (Meissner *et al.,* 2007). It was investigated that several genes including *SOD1*, *DPP6*, *ITPR2*, and *TARDBP* are involved to ALS generally presents between (Mauritz *et al.,* 2008). An ALS iPS cell model showed the multigenic nature of this disease. Dimos *et al* experimentally generated iPS cells from a skin sample taken from an elderly patient with familial ALS displaying a mutation in *SOD1* (Viswanathan *et al.,* 2008). The number of motor neurons generated from the ALS iPS and control cell lines were not reported in this study that will be very helpful for further study.

Drug development

The drug development process initiates with the patient samples collection for the generation of induced pluripotent stem cells (iPSCs) (Utikal *et al.,* 2009) , followed by directed differentiation of these cells into cells that have a crucial role in the disease. The characteristic of the technology that makes it valuable for drug discovery is the capacity to recapitulate crucial aspects of the disease for drug screening (Vierbuchen *et al.,* 2010). A schematic diagram of the iPSC production process is shown in Figure 3. (Modified from Maherali and Hochedlinger, 2008)

iPSCs in cell therapy

There are several kinds of obstacles in the process of organ transplantation especially in the case of non related individuals (Mayer *et al.,* 2000). Besides it may have severe side impact for life long treatment using several highly powerful drugs. Inspite of having limitation to use human embryos for donor issues, it has chance to open a new era to use iPSCs inorder to serve as custom-tailored replacement cells in a therapeutic setting. At first Zhao and his colleagues experimentally showed that teratomas derived from some syngeneic iPSCs elicit an immune response from the host animal (Xu *et al.,* 2008). Another group of scientists used the method of gene targeting in order to correct the mutation in iPSCs those were collected from sickle cell anemia model animal(Mikkelsen *et al.,* 2008). When these cells were transplanted into an irradiated mouse that caused a reversal of the defected phenotypes. In 2008, Wering *et al.*, demonstrated that transplantation of iPSC derived Dopaminergic

neurons into a mouse that was affected by Parkinson's disease, was sufficient to restore neuronal function. A related approach was demonstrated with human patients with Fanconi's anemia (Tsubooka *et al.,* 2009). In this case, the mutant gene was replaced using lentiviral vectors prior to reprogramming of the patient's fibroblasts and keratinocytes, as the genetic instability of the mutant fibroblasts made them nonpermissive for iPSC generation. Significantly, these iPSCs could be differentiated into hematopoietic progenitors as efficiently as ESCs (Yang *et al.,* 2009) and wild-type iPSCs and capable to maintain the disease-free phenotype *in vitro* (Melton *et al.,* 2010).

Personalized treatment

Hopefully it will be the most effective use of iPS cells. Actually personalized drug is such kind of drug that will only develop for single individual as well as depends on the genetic information of him or herself. There are some obstacles to reprogram in order to generate iPSC. When it would be possible to generate iPSC from individual then that could be used to screen drug for them (Mikkola *et al.,* 2002). Though theoretically it is possible but in practically it has to face some difficulty. The ancestral human disease is habitually associated to distinct mutations in individual genes (Caspi *et al.,* 2008). Accurate correction of this genetic fault in patient-derived stem cells and iPSCs is a significant difficulty to the extensive purpose of tailored cell-based therapy. It has investigated that Zinc finger nuclease (ZFN) technology has emerged as a highly resourceful innovative tool for accurate eukaryotic gene editing directly at the endogenous genomic locus (Blelloch *et al.,* 2008). At first Collin and Lako applied the ZFNs to genome editing in human iPSCs that ensured positive signal for cell-based therapy (Meyer *et al.,* 2009). Individual patient-derived iPSCs are providing new opportunities to modeling human disease *in vitro* (Mayshar *et al.,* 2010). Another researcher group used ZFN-based genomic editing to generate isogenic sets of human disease and control pluripotent stem cells that differ solely in the α-synuclein gene (Ebert *et al.,* 2008). If it is possible to develop personalized drug successfully, all of the side effects associated with drug will be lessen (Young *et al.,* 2011).

iPSCs in toxicological and pharmacological screening

Recently iPSC has emerged as an awaiting implement for pharmacological and toxicology screening (Moehle *et al.*, 2007). We know that adverse drug reactions represent a major confront for pharmaceutical industries, hospitals and drug regulators as well as are major contributors to the high cost of drug development (Tsuji *et al.*, 2010). In addition to currently utilized toxicology assay has several type problems based on established cell lines, primary explanted somatic cells and laboratory animals (Mollamohammadi *et al.*, 2009) .The development of predictive human cellular systems that complement current toxicity tests in animals and primary cells are therefore vital. Stem cells utilized for toxicology screening can be of adult, fetal or embryonic origin (Ying *et al.*, 2008). However, the capacity of human embryonic stem cells (hESC) to be propagated within in vitro culture covers a distinct advantage over primary cultures of fetal and adult stem cells as hESC lines are hypothetically immortal. Yu *et al.* and Takahashi *et al.* demonstrated (Yu *et al.* 2007) a new opportunity for toxicology assay development that is free of ethical and moral controversy. But there are some barriers to utilize iPSC for toxicology screening assays. First of all it is unknown how the epigenetic state of reprogrammed iPSC actually compares with hESC derived from 'normal' human blastocyst-stage embryos (Di *et al.*, 2008). There is a chance that restrained divergences in the epigenetic programming of iPSC. The second major barrier is that the derivation of iPSC entails permanent genetic modification to somatic cells (Wernig *et al.*, 2008), due to the use of viral transduction of recombinant DNA (Markoulaki *et al.*, 2009). Hopefully, today or tomorrow the use of iPSCs to personalize drug development may prove to be powerful resources of plummeting drug toxicity, stratifying patient response and reducing late-stage

clinical failures (Lapillonne *et al.*, 2010; Marica *et al.*, 2011).

Vaccination

The recent study on cell based vaccination using transplantation of iPSC-derived memory B cells (Li *et al.*, 2009) has initiated a new era for vaccination. Li *et al.*, first induced somatic cells to form iPSCs and expanded (Monzo *et al.,* 2006). Next the cells were genetically or chemically promoted to an immune cell fate, followed by *in vitro* antigen-presenting and -processing procedures to produce memory B cells (Judson *et al.,* 2009) that could secrete functional antibodies to different pathogens. Finally these cells were transplanted back into a human (Li *et al.*, 2009). This study provided a positive signal to develop vaccine via iPSCs (Tao *et al.,* 2010; Tchieu *et al.,* 2010).

Challenges and future perspectives

Despite the fact that iPSCs offer unparalleled potential for Medical Biotechnology including disease research, drug screening, toxicology, regenerative medicine, vaccination etc., and this technology will be fittest when researchers will have capability to overcome all of the challenges or barriers related to the methodology (Yoshida *et al.,* 2009). First of all, theoretically iPSC can give rise to all somatic cell types (Hou *et al.*,2006) , but practically, in vitro differentiation protocols to date have been developed for only some specific cell types (Rai *et al.*, 2008; Schenke *et al.*, 2008). In many experiment, insufficiency of differentiation have been producing cultures with various type of cells for last decades that is vital challenge to the researchers of the field related to iPSC technology. Second, it is not possible yet to develop an active cryopreservation method that ensure support in storage and transplantation (Kaji *et al.*, 2009; Taura *et al.*, 2009). Third, not only integrating viruses induces potential mutations, ultimately tumours in the case of therapeutic applications, but also undifferentiated iPSCs themselves would be tumorigenic as donor cell grafts would be contaminated by pluripotent undifferentiated cells (Fusaki *et al.,* 2009; Guenther *et al.,* 2010). Fourth, the safety concern is another major constraint for transplanting cells into human patient (Klapstein *et al.*, 2001; Pereira *et al.*, 2010). Fifth, maximum patient-specific iPSCs have been generated with integrating vectors, which could disrupt endogenous genes during cell therapy (Jia *et al.,* 2010). Sixth, inefficient targeting approaches may cause karyotypic abnormalities due to extensive culturing n the case of diseases requiring gene targeting in order to repair mutant alleles (Ellis *et al.,* 2010). Seventh, reprogramming is particularly challenging as the genome-wide epigenetic code must be reformatted to that of the target cell type in order to fully reprogram a cell (Morizane *et al.,* 2009). Finally, patient specific iPSCs needs to be derivated from diseased tissue portions (i.e. hepatocyte within liver cancer) rather than the tissues which do not carry any pathogenetic events (i.e. skin fibroblasts for liver cancer) (Bussmann *et al.*, 2009). In near future possible it can be possible to use iPSC technology in order to treat other diseases. Researcher also demonstrated that iPSC has high telomerase activity that is linked to ageing. So we hope, one day it will be used as a tool for ageing research as well as for Medical Biotechnology.

Conclusion

iPSCs have supreme potentiality for Medical Biotechnology including disease research, drug screening, toxicology and regenerative medicine etc. though the process of reprogramming is ineffective and often deficient. But it is a matter of wonder that this technology is hurriedly emerging day by day due to the importance of demands. The innovation of iPSCs has also predisposed the attention of researchers as the activation of only a few transcription factors can transform cell fate by simple steps. We envisage that such kind of technology will be able to overcome all of the challenges behind efficient implementation as well as will lead to new insights into various kind of illness in favor of Medical Biotechnology.

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