Review Article

Applications of Induced Pluripotent Stem Cells in Clinical Practice

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Abstract

Stem cell research has shown a promising future in understanding and treating diseases from cellular level, factoring minimum patients' suffering in terms of toxicity and persistent or endless therapy. Induced pluripotent stem cells offer a variety of opportunities that were unimaginable in the past. Developments and breakthroughs from this field of science are constantly evolving with quite remarkable or rather overwhelming articles published. This is a systematic review study with the aim to simplify the basic understanding of induced pluripotent stem cells. Its journey so far and prospects to confer the solutions in clinical practice.

Keywords: Cells, pluripotent, stem

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Introduction

The human body has always been a fascinating structure. The more we try to understand it, the more mysterious it becomes. Vigorous work is being done worldwide to unravel this complex puzzle. One of the astonishing parts of the human system is the cell. Since the discovery of the cell by Robert Hooke centuries ago, it has been unanimously agreed that the cell is the building block of life.

Cells have been utilized in many ways which include understanding pathological, healing, and therapeutic processes of the living system. Recently, the cell itself is being considered a form of therapy due to the discovery of its ability to "shape shift" or differentiate into a desired cellular configuration. These types of cells are called stem cells.

Stem cells are broadly classified into embryonic stem cells (ESCs) (derived from an early stage of the embryo) and adult stem cells (also called somatic cells, which maintain and repair tissues which they are found). These could be further classified into totipotent, pluripotent, and multipotent stem cells due to their levels of differentiation.

Pluripotent cells can differentiate into all types of somatic cells,[1] but the main challenge was to directly convert the somatic cells into pluripotent cells which could be used for the regeneration of damaged or diseased cells. This wasn't possible until 2006 when for the first time Shinya Yamanaka's group successfully induced embryonic stem (ES)-like cells from mouse fibroblast cells and named them 'Induced pluripotent stem cells' (iPSC).[1,2] A year later, the same group and another group[3] successfully generated human iPSCs through the direct programming of defined transcription factors that are highly expressed in pluripotent cells.[1-3] This earn them a Nobel Prize for their combined efforts in reprogramming mature cells into embryonic cells in 2012.[4] The aim of this study is to simplify the understanding of iPSCs, the journey so far and prospects to confer solutions in clinical practice.

Methodology

Study design

This was a systematic review study.

Search strategy

Literature search was carried out to collect the multiple research studies and papers through the list of NCBI databases and citation indexes in Web of Science, Update and PubMed following a critical appraisal [Table 1 and Figures 1 and 2].

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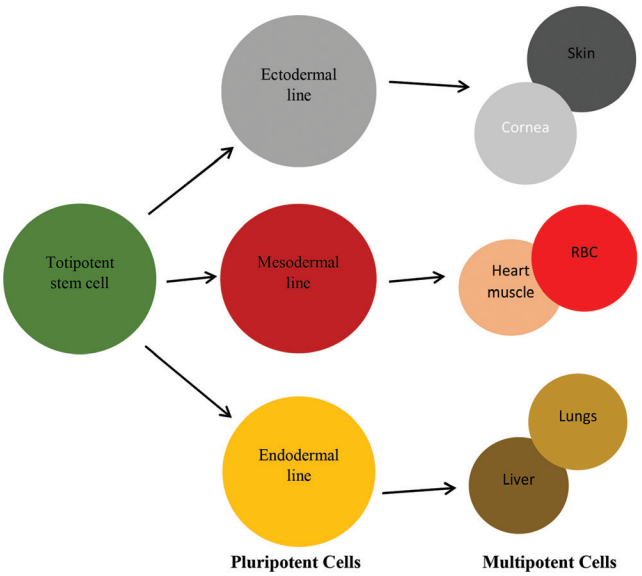

Figure 1: Types and differentiation of stem

Inclusion criteria

- Relevant studies from 2000
- Studies presented in English
- Studies with access to full text
- Online websites with relevant information.

Exclusion criteria

- Records/studies with inconclusive results.

What are Induced Pluripotent Stem Cells?

Pluripotency is a biological term with a Latin origin. "Plurimus" means very many and "potens" means having power.[5] Therefore, pluripotent simply means having the power to be very many. With the above background knowledge, it will be easier to now explain what iPSCs are.

When somatic cells are harvested from blood, hair, skin, and renal epithelial cells in urine and are reprogrammed through "forced" expression of certain genes and transcription factors to exhibit a high differentiative characteristics similar to ESCs, they are now referred to as iPSCs.[2,4,6,7] Reprogramming process is a meticulous, technically demanding and tedious exercise requiring an advanced state-of-the-art laboratory facility.[6]

Generation of induced pluripotent stem cell

Over the years, reprogramming of somatic cells into iPSC has been done through various methods. These include somatic nuclear transfer, cell fusion, reprogramming through cell extracts, and finally, direct reprogramming which is regarded as the breakthrough and this is the gold standard for the generation of iPSC. Direct reprogramming has widely been researched will be the focus of this study Figure 3.[7]

Direct reprogramming

This is a method of reprogramming cells which involves the introduction of transcription factors called Yamanaka or Thomson factors through viral or nonviral vectors system to subvert the somatic cells into pluripotency. The molecular mechanisms underlying somatic cell reprogramming to pluripotency for the creation of high-quality pluripotent cells is a complex and critical process.[8,9]

Cellular harvesting

The process of cellular harvesting varies with the somatic cell of interest. It is an important step in cellular reprogramming. Keratinocytes, for example, could be derived from plucked hair which is then cultured in flasks until the outgrowth of keratinocytes is visible.[10] T-cells have also been used in the generation of iPSC which are harvested from blood through venipuncture, processed and activated T-cells are derived through the meticulous process of centrifugation, separation, and culture which takes a minimum of 10–14 days.[11]

Reprogramming

Naturally, not all cells are the same, and therefore, the ease of reprogramming differs from cell to cell. This brings about the classification of cell for reprograming from easy to hard [Figure 4].[12] The reprogramming stage involves the transduction of prepared harvested cells with transcription factors (Yamanaka or Thompson factors) using a vector. There are two types of vectors used for the delivery of transcription factors: integrative and nonintegrative[Figure 5 and Table 2].[12]

Integrative vectors incorporate the transcription factors into the cell genome which ultimately results in remodeling of epigenetic markers which changes the activity of a DNA segment but not the sequence; that is a change in phenotype without a change in genotype.[13] The nonintegration also called integration-free method achieves cellular reprogramming without change in epigenetic make-up of the cells and this conserves the original DNA [Figure 2].[14]

Differentiation

Induced pluripotent stem cell colony identification and isolation

Colonies of iPS-like cells to emerge days after transferring onto feeder cells. The iPSC colonies are then isolated and plated in feeder cells.[15-17] The growth of stem cells in culture requires certain nutrients that support the cells in an undifferentiated state, these are called feeder cells (example include; Mouse Embryonic Fibroblasts-MEF Feeder Cells, JK1 Feeder Cells-an immortalized CD34+ testicular stromal cell line which supports long-term proliferation of numerous types of stem cells and SNL 76/7 Feeder Cells are clonally derived from a mouse fibroblast STO cell line).[18]

Characterization of induced pluripotent stem cell

When iPSCs are ready for picking, they could be transferred to a feeder-free medium. Differentiation is observed with staining of the cells (with substances like Tra-1-81 antibody) and characterized into various stages of differentiation. Some transcription factors such as Oct-4 has been used for staining.[14] Prolonged incubation has been observed to

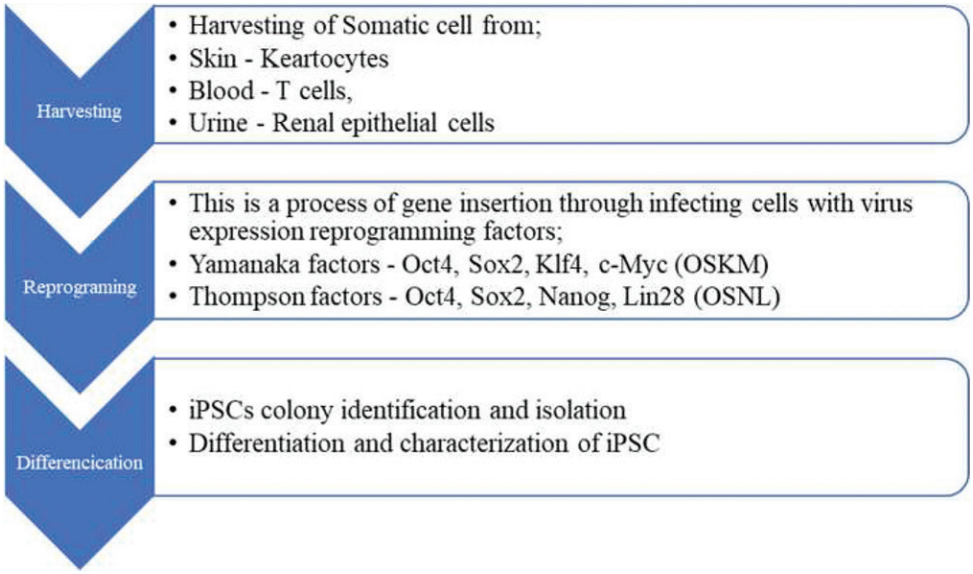

Figure 2: Prisma Flow chart

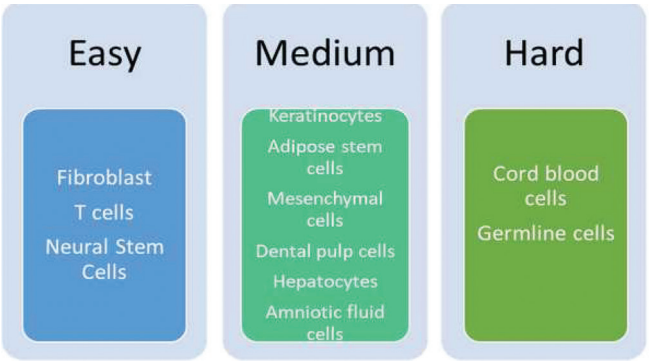

Figure 3: Steps in direct reprogramming method

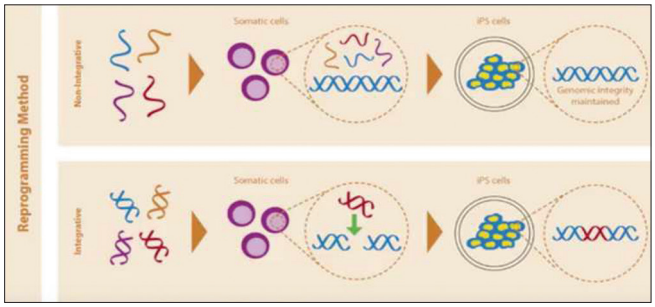

Figure 4: Classification of cells based on ease of reprogramming

encourage the rapid differentiation of iPSCs.[17] Established iPSC clones should be tested using immunocytochemistry for the expression of pluripotency markers, and a detailed molecular characterization of the generated iPSC lines should be done. This should include the analyses of the pluripotency gene expression, demonstration of the DNA demethylation at the promoters of pluripotency genes, and analyses of the transgenes silencing.[17]

Clinical Applications of Induced Pluripotent Stem Cell

Disease modelling

Diseases therapy could be difficult because of the lack of information about the disease processes and progression. This is particularly important given many retinal diseases do not have a comparable animal model or do not manifest in mice, etc., or caused by an unknown gene. Access to affected diseased human retina is difficult or impossible, unlike other tissues where biopsy could be taken, and different types of analysis done for better understanding of disease process and therapeutic processes. This area is the most active at the moment because it allows us to set up the assays to not only understand disease but also to screen human cells against various pharmacological, etc., products to ascertain their efficacy in treating a specific disease.

Human iPSCs have brought about ways these diseases need to be modeled so that treatment could be developed aiming to target the main mechanism involved. Furthermore, human iPSC derived models may also be useful to bridge the gap between preclinical animal studies and clinical trials.

iPSC-derived retinal pigment epithelial (RPE) cells with MER Receptor Tyrosine Kinase (MERTK) an enzyme that mediates the phagocytosis of apoptotic cells and modulates cytokine production. Deficiency to MERTK creates an accurate *in vitro* model of human disease is now possible.[18] MERTK-RPE cells with defective engulfment of photoreceptor outer segment have been modeled using the translational read through inducing drugs G418 and PTC124. It may be possible, therefore, to use TRIDs to treat retinitis pigmentosa (RP) due to nonsense variants in MERTK, which hitherto has no effective therapy.[19]

Photoreceptors with rudimentary outer segment are also available.[20] This is an important advancement because it can be used to understand the disease mechanisms and as an important model to test potential new therapies for inherited retinal ciliopathies.

Diseases due to deficiency such as adenosine deaminase deficiency-related severe combined immunodeficiency, Shwachman-Bodian-Diamond syndrome and have been studies and were concluded that they are inherited in a classical autosomal-recessive Mendelian Inheritance manner like congenital disorders. They also lead to the understanding that these diseases are a result of point mutations in those genes which were vital for normal hematopoiesis and immunological function. Other disease studied so far are; Duchenne muscular dystrophy and Becker muscular dystrophy, Parkinson disease (PD), Huntington disease, juvenile-onset, type 1 diabetes mellitus, Down syndrome (DS)/trisomy 21, and the carrier state of Lesch-Nyhan syndrome.[21]

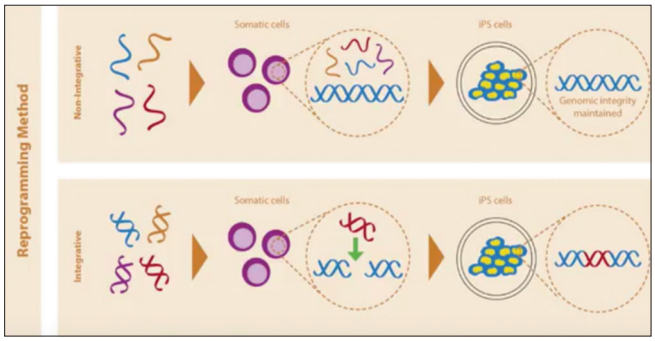

Figure 5: Reprogramming method

Table 1: Search Strategy

Database	PubMed
Date	01/06/2017
Simple Search	
Prospects of induced pluripotent stem cells in management of retinal degenerative diseases	
Advanced Search	
Search Strategy 1	**#1 AND #2 AND #3 AND #4**
#1	Induced pluripotent stem cells OR iPSCs OR Human induced pluripotent cells OR HiPSCs or hiPSCs
#2	Retinal degenerative diseases
#3	Prospects OR Future OR Potentials
#4	Management OR Treatment OR Clinical trials

iPSCs in combination with microarray and RNA sequencing technology were used for the identification of molecular networks that drive the different aspects related to the pathogenesis in Down's Syndrome through the generation of phenotype-genotype maps of complex diseases by linking various defects with phenotypes, like in Down's syndrome using chromosome engineering of DS-iPSCs.[21]

PD is a very common neurodegenerative disease, in which treatment has not been possible due to the reason that by the time, PD gets clinically manifested, the neurons have already lost, which makes it very difficult to be able to study the underlying mechanisms of PD to develop a treatment of it. In such a situation, iPSCs can be used and experiments are being carried out in this aspect.[21]

Regenerative medicine or gene therapy

Injured or degenerated tissues are repaired by the replacement of those tissues with iPSCs generated from the somatic cells from patient's own body and transplanting them to the site of injury or degeneration. Conditions that can be treated include hematopoietic disorders, musculoskeletal injury, spinal cord injury, and liver damage by the generation of specific cells with the help of iPSCs. Degenerative diseases in which cells death are the main mechanism that are being studied through

Table 2: Integrative versus nonintegrative method of reprogramming[2-10]

Method	Integrative	Nonintegrative (Integration free)
Vectors	Retrovirus	Adenovirus
	Lentivirus	Sendai virus
	PiggyBac system	mRNA
		Expression plasmids
		Episomal vectors
		Minicircle vector
		Liposomal magnetofection[15]
Advantages	Suitable for a variety of cells	Suitable for a variety of cells
	Delivery of large genetic elements without reduction in efficiency[7] (specific to PiggyBac systems)	Conserves original DNA hence better for clinical trials
		Higher efficiency of up to 1% and more
		Faster rate of reprogramming
Disadvantages	Risk of infections and teratomas	Multiple transfection is required (as frequent as daily)
	Genetic alteration (may not be permanent in some)	Expensive
	Not suitable for clinical applications	High biosafety (especially with Sendai virus)
	Storage and quality control difficulty	Difficulty in controlling the gene expression level (especially for adenovirus)[16]
	Relatively longer duration of reprogramming without control of time and dose (12)	
	Efficiency as low as 0.1% (especially with retrovirus)	

gene therapy using iPSCs. One such disease is RP where iPSCs generated from the patient suffering with the disease were shown to differentiate into rod photoreceptor cells.[22] The differentiation of iPSCs to RPE is proved to be beneficial for the patients of retinal pigmentosa and age-related macular degeneration.[21]

Drug discovery and cytotoxicity studies

Another significant application iPSCs are their complementarity to drug discovery or prediction of toxicity through animal models.[21] Although animals or *in-vitro* animal derived cells are used for testing new drugs, their ability to replicate human physiological conditions and related phenotypic attributions is limited. It has also been demonstrated that the results derived from animal models testing do not come out to be beneficial in humans. The superoxidase dismutase gene associated with amyotrophic lateral sclerosis is a good example. It allowed for the identification of Vitamin E and creatine to be relievers of the diseased phenotype which was a breakthrough but it failed to achieve improvements in humans.[21] Hence, studies need to be carried out which will include the identification of a potential drug molecule followed by its synthesis, generation of iPSCs, their differentiation to specific somatic cells, and testing for toxic or nontoxic effects of the synthesized drug on the somatic cells in a system where the results could be directly extrapolated to humans.

The use of iPSCs in the development models that predicts toxicity and side effects more accurately before clinical trials may help to reduce the cost by demonstrating cardiotoxicity or hepatotoxicity caused by the drugs much before they reach clinical trials is proving invaluable. This will further reduce the duration taken for clinical trials of drugs which will eventually fail due to cytotoxicity in the later stages.

Conclusion

Imagine a world where diseases could be cured with one's own cells. Individuals are free form dealing with life-long diseases, numerous side-effects of drugs and failures, having to endlessly wait for a match to have an organ/tissue transplant and so much more. These are the potentials we envisage for iPSCs.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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