Clinical Cancer Investigation Journal-ISSN: 2278-0513

Regenerative Medicine with iPSC Cells: Generation, Application, and Ethical Issues

Bozorgmehr Norouzi **(first author)**
General practitioner, Guilan Road Trauma Research Center, Guilan University of Medical Sciences, Rasht, Iran

ORCID: 0000-0001-7137-7460

Email: B.noroozi91@gmail.com

Sahar Heidary

Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran.

ORCID: 0000-0003-1010-8456

Email: Sahar.heidary.sh@gmail.com

Farzane Iranmanesh

General practitioner, Guilan Road Trauma Research Center, Guilan University of Medical Sciences, Rasht, Iran

ORCID: 0000-0002-7295-3797

Email: Farzaneiranmanesh9270@gmail.com

Mahroo Mohammadhosseini

General Practitioner, Medical School, Azad University, Tehran, Iran

ORCID: 0000-0001-5061-1182

Email: Mahroomohammadhosseini@gmail.com

Danial Nikfar

General practitioner School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

ORCID: 0000-0001-5754-9019

Email: danialnikfar32@gmail.com

Amirhossein Roshan

Student Research Committee, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

ORCID: 0000-0001-7623-141X

Email: Roshanamir962@gmail.com

Bahar Alizadeh

School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

ORCID: 0000-0003-2569-7150

Email: Bahar_93072@yahoo.com

Pourya Medhati

Student research committee, Shiraz University of Medical Sciences, Shiraz, Iran

ORCID: 0000-0001-6684-3439

Email: Pourya Medhati@gmail.com

Arass Rasaei

Student research committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Nasim Athari Boroujeni **(corresponding author)**

Neurologist, Neuroscience Research Center, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

ORCID: 0000-0002-5676-0805

Email:Nasimathariboroujeni@gmail.com

Abstract

Despite medical advances, many diseases still exist without suitable treatments worldwide. Incurable disorders are treated through techniques that decrease bodily and psychological symptoms. Management rather than treatment is the approach physicians take in such circumstances. Researchers are developing new treatment methods to control symptoms and treat diseases without a cure. In modern science, regenerative medicine is a promising treatment option for illnesses that are currently untreatable. In regenerative medicine, there is a new and promising approach to treating patients whose illness has limited or no other treatment options. Regenerative medicine considers induced pluripotent stem cells (iPSCs) a valuable resource for replacing diseased or damaged tissues since they can be made from any healthy human or patient. Further, reprogramming technology has enabled the study of cell fate decisions as well as the modeling of human diseases, significantly increasing the chances of (i) discovering new drugs through screening formats as well as (ii) treating life-threatening diseases with cell therapy. This review aims to familiarize the reader with the application of iPSCs in different fields of medicine. Due to promising in vivo, in vitro, and pre-clinical findings, many studies have been conducted using these stem cells to treat various conditions. This review aims to familiarize the reader with the application of iPSCs in different fields of medicine.

Keywords: *Regenerative Medicine, Stem cells, Ethic, Pluripotent*

Introduction

1. Introduction

Despite medical advances, many diseases still exist without suitable treatments worldwide. Incurable disorders are treated through techniques that decrease bodily and psychological symptoms. Management rather than treatment is the approach physicians take in such circumstances. Researchers are developing new treatment methods to control symptoms and treat diseases without a cure. In modern science, regenerative medicine is a promising treatment option for illnesses that are currently untreatable (1).

Several disciplines are incorporated into regenerative medicine, including cell biology, genetics, biomechanics, material science, and computer science (2); the goal is to restore normal function to injured or degenerated cells or tissues (3). It has been recognized that stem cells can be used to repair organs and tissues since they were discovered and their unique properties. The potential applications of these cells in regenerative medicine make them a good candidate for this field (4). Researchers studying regenerative medicine consider it an alternative to traditional drug-based therapies in various diseases, including degenerative diseases (5–8).

Using induced pluripotent stem cells (iPSCs), differentiated somatic cells can now be converted into multipotent stem cells capable of generating all types of adult tissues. Consequently, this technology has various applications, including regenerative medicine, in vitro disease modeling, and drug discovery (9,10). For reprogramming, expansion, isolation, and differentiation of iPS cells, bioengineering technologies offer novel tools through their application to biological and biochemical techniques. Regenerative medicine considers iPSCs a valuable resource for replacing diseased or damaged tissues since they can be made from any healthy human or patient. Further, reprogramming technology has enabled the study of cell fate decisions as well as the modeling of human diseases, significantly increasing the chances of (i) discovering new drugs through screening formats as well as (ii) treating life-threatening diseases with cell therapy. This review aims to familiarize the reader with the application of iPSCs in different fields of medicine.

2. Regenerative Medicine

Cell regeneration is the central concept of regenerative medicine, and different cells have been used to accomplish this goal. Several studies, however, have shown that cell therapy has some limitations. In recent years, other alternatives have been introduced for cell therapy to resolve these limitations. These alternatives include the improved application of stem cells for the restoration of tissue, such as the combination of cells with scaffolds, cell cultures with suitable biochemical properties, gene editing, and the immunomodulation of stem cells, as well as the use of stem cell derivatives (11,12). however, the use of these alternatives clinically may be postponed, as more pre-clinical studies are required due to their status as newer technologies (13). Based on regenerative applications, stem cells can be categorized as embryonic stem cells (ESCs), tissue-specific progenitor stem cells (PSCs), mesenchymal stem cells (MSCs), umbilical cord stem cells (UCSC), bone marrow stem cells (BMSCs), and induced pluripotent stem cells (iPSCs) (10).

3. History of iPSCs

By introducing pluripotent-associated genes into adult somatic cells, iPSCs can be defined as "embryonic stem cell-like" cells. The most well-known pluripotent stem cells were ESCs, derived from the inner cell mass of a blastocyst of a preimplantation embryo. Similar to ESCs, iPSCs can differentiate into the three germ layers of endoderm, mesoderm, and ectoderm in culture. In mouse embryonic stem cells, Takahashi and Yamanaka set out to identify genes involved in maintaining pluripotency. After searching for reprogramming factors related to ES-cell pluripotency, they identified 24 candidates. The ability to induce pluripotency was tested using a screening method developed for 24 pluripotency-associated candidate factors. Using a retroviral delivery system, these genes were transduced into mouse embryonic fibroblasts (MEFs). To generate mouse fibroblasts, the mouse F-box only protein 15 (Fbxo15) gene locus was fused with a β-galactosidase (β-geo) cassette. The expression of β-geo is used as a reporter of Fbxo15 expression and activity, as cells expressing β-geo are resistant to the selection marker geneticin (G418). Normal somatic cells are not immune to G418 treatments and do not express the Fbxo-15 locus. The Fbxo15-β-geo MEFs were used to screen the pool of 24 transcription factors by transducing different combinations of the candidate genes and assessing the capability of the MEFs to survive in G418 treatment. The result of successive rounds of elimination of each factor was the identification of four core genes, namely Oct3/4, Sox2, Klf4, and c-Myc (OSKM cocktail/factors) (14). After injection into immunocompromised mice, the reprogrammed cells exhibited ES cell-like morphology, expressed ES cell marker genes such as SSEA-1 and Nanog, and formed teratomas (14). By ectopically expressing defined transcription factors, Takahashi and Yamanaka were able to reprogram mouse fibroblasts back to a pluripotent state, circumventing the ethical concerns surrounding ESCs. In contrast, these "first generation" iPSCs failed to generate adult chimeras or contribute to the germline due to a low expression level of crucial ES pluripotency genes (14), thus suggesting that the iPSCs were only partially reprogrammed. Using modified induction protocols, Yamanaka and other laboratories produced iPSCs capable of making adult chimeras and transmitting germlines in 2007 (15).

4. Induction of pluripotency in differentiated cells

This subject means roll-back in the development field to get stem cells from cells that have been totally or partly differentiated. In this field, inspiring results have been obtained. Up to now, induced pluripotent stem cells (IPSCs) have been derived from human and mouse somatic cells. Though these cells develop well in laboratory settings, tests that are done in natural conditions do not always provide acceptable results; so IPSCs cells can be transplanted to different target organs, but they create the ability to reproduce and differentiate in some parts of the organ (not all parts) (16). Furthermore, regarding their ability to create each of the germ layer cells, transplantation of these cells can be associated with a high rate of tumor formation in organs. A successful development based on an efficient and reliable process of cell dedifferentiation will provide a way to replace failed transplants and provide life expectancy for thousands of people worldwide. This article aims to bring about a general review of the production of induced pluripotent stem cells, which constitutes the most important and promising dedifferentiation methods used in different research groups worldwide. Different differentiated cells have been used in animals to produce induced pluripotent stem cells.

5. Methods of inducing pluripotency

For nearly ten years, research protocols related to this issue have been extensively developed, while Evans and Kauffman conducted the first studies on the production of embryonic stem cells about 30 years ago (17). Diverse human, rat, and mouse cells have been used to analyze the genes involved in the differentiation process and maintain the state of pluripotency. Little is known about the mechanisms of reprogramming or dedifferentiation, but it is evident that chromatin rearrangement is a key step in this process. After the dedifferentiation process is completed, Wnt, LIF, and BMP signaling pathways, which are a group of proteins signal transduction pathways and direct signals from the outside of the cell to the inside of the cell through cell surface receptors, are involved in the process of self-renewal to maintain stem cells (18). The dedifferentiation process can be evaluated in dissimilar stages. During this process, the expression of the desired cell's genes is reversed, and as a result, on the one hand, the activity of genes related to cell development is deactivated, and on the other hand, genes related to dedifferentiation are activated. At the protein level, there are also variations in expression, which comprise more expression of progenitor cell proteins and less expression of proteins related to differentiated cells. In this process, the morphology of the cells is also affected, during which the undifferentiated cells appear smaller than the differentiated cells. In terms of karyoplasm and organelles, they have a higher and a lower amount, respectively, compared to the differentiated cells. At functional levels, undifferentiated cells have more ability to become a wide variety of cells than differentiated cells (19). Nevertheless, the problem arises when the rescheduling method has to be selected. There are several ways to restore differentiation, but none can do so without the use of viral

vectors, cell conjugation, or guaranteed safety, like in critical situations.

6. Applications of iPSCs

It is expected that iPSCs will have a significant impact on regenerative medicine, the pharmaceutical industry, and animal biotechnology. It is possible to utilize human iPSCs for curative treatments, investigate disease onset and progression in vitro, and test potential therapeutics in high throughput screens. iPSCs are being used to treat a variety of conditions, as illustrated in Table 1, and they are being used to model pathogenesis in a variety of conditions, as shown in Table 2.

Table 1. Application of iPSCs in the treatment of different conditions

Disease	***study***	***filed***	***Results***	***year***	***ref***
Parkinson's disease	*In vitro*	Neurology Psychiatry	The iPSCs functioned as midbrain dopaminergic neurons.	2020	(20)
GVHD	*Clinical trial*	Internal medicine	iPSC-derived MSCs cause complete response and overall survival.	2020	(21)
Parkinson's disease	*In vitro*	Neurology Psychiatry	The animals show behavioral improvement.	2020	(22)
GVHD	*In vitro*	Internal medicine	By administering Cymerus™ iPSC-MSCs, disease severity was reduced, and survival was prolonged.	2019	(23)
Limb ischemia	*In vitro*	General Surgery	Following iPSC-EC transplantation, neovascularization of the ischemic limb is achieved through arteriogenesis.	2018	(24)
Degenerative joint diseases	*In vivo* *In vitro*	Orthopedic Surgery	hPSC-derived chondrogenic progenitors can form stable cartilage tissue *in vitro* and *in vivo*.	2015	(25)
Age-related macular degeneration	*In vitro*	Ophthalmology	As measured by the optokinetic response, i-NPC treatment completely preserved visual acuity.	2015	(27)
HIV	*In vivo*	Internal medicine	Upon viral challenge, the anti-HIV iPSC-derived macrophages exhibited strong protection from HIV-1 infection	2011	(32)

Table 2. Application of iPSCs in modeling pathogenesis of the different condition

Disease/Organ	***field***	***Results and Conclusion***	***year***	***ref***
Autism Spectrum Disorders (ASD)	Psychiatry	ASD-derived organoids exhibit an accelerated cell cycle and overproduction of GABAergic inhibitory neurons	2015	(35)
Stage-dependent human liver disease	Internal medicine	Using iPSCs generated from a cohort carrying mutations (PiZZ) in the gene responsible for alpha-1 antitrypsin (AAT) deficiency, it was found that the global transcriptomes of PiZZ iPSCs diverge from normal controls upon differentiation to hepatic cells. The expression of 135 genes distinguishes PiZZ iPSC-hepatic cells, providing potential clues to liver disease pathogenesis.	2015	(36)
Hypertension	Internal medicine	It was demonstrated that established HTN iPSCs could robustly and reproducibly differentiate into functional vascular smooth muscle cells (VSMCs), a cell type most relevant to vasculature tone control. Moreover, a sensitive traction force microscopy assay demonstrated that iPSC-derived VSMCs show a quantitative contractile response	2015	(37)

		to the physiological stimulus of endothelin-1. Furthermore, the inflammatory chemokine tumor necrosis factor α induced a typical VSMC response in iPSC-derived VSMCs.		
Human lung organoids (HLOs)	Internal medicine	By manipulating developmental signaling pathways, hPSCs generate ventral-anterior foregut spheroids, which are then expanded into HLOs. HLOs consist of epithelial and mesenchymal compartments of the lung, organized with structural features similar to the native lung.	2015	(38)
Ischemic damage in the aldehyde dehydrogenase 2 genetic polymorphism	Internal medicine Pediatric	induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) generated from individuals carrying the most common heterozygous form of the ALDH2*2 genotype. These results reveal a new function for the metabolic enzyme ALDH2 in the modulation of cell survival decisions. Insight into the molecular mechanisms that mediate ALDH2*2-related increased ischemic damage is important for the development of specific diagnostic methods and improved risk management of CAD and may lead to patient-specific cardiac therapies.	2014	(39)
Fanconi anemia	Internal medicine Pediatric	A drug-screening platform model was validated by identifying several compounds that improve the hematopoietic differentiation of FA-iPSCs.	2014	(40)
Retinitis pigmentosa	Ophthalmology	In this model, a reduction was found in survival rate in the photoreceptor cells with the E181K mutation, which was correlated with the increased expression of endoplasmic reticulum (ER) stress and apoptotic markers.	2014	(41)
Multiple Sclerosis (MS)	Neurology	Despite some electrophysiological differences between MS-iPS-derived neurons and control cells, they displayed functional properties, including robust resting membrane potentials, enormous fast tetrodotoxin-sensitive action potentials, and voltage-gated sodium currents. The results of this study demonstrate for the first time that disease cell lines derived from skin cells obtained from an MS patient can be generated and successfully differentiated into mature neural lines.	2012	(25)
β-thalassemia	Internal medicine Pediatric	In this study, human embryonic stem (hES) cells and human iPS cells demonstrate high correlation coefficients. According to this study, human amniotic fluid cells may provide a rapid and efficient way to generate patient-specific iPS cells.	2012	(42)
Familial Hypercholesterolemia (FH)	Internal medicine Pediatric	It has been shown in this study that (1) FH-iPSCs can be used to produce hepatocytes; (2) FH-iPSC-derived hepatocytes do not uptake LDL-C as efficiently as control cells; (3) control hepatocytes increase LDL uptake when lovastatin is given; and (4) FH-iPSC-derived hepatocytes secrete lipidated apolipoprotein B-100 in marked excess.	2012	(43)
Huntington's disease (HD)	Pediatric Neurology	A vacuolation phenotype was observed in astrocytes derived from HD patients' iPSCs in this study. By modeling HD and screening high-throughput therapeutics with human iPSCs, future mechanistic investigations can be carried out.	2012	(44)

Schizophrenia	Psychiatry	Researchers found that SCZD h-iPSC neurons were less connected, had fewer neurites, and expressed lower levels of PSD95-protein. Several cyclic AMP and WNT signaling pathways were altered in SCZD hiPSC neurons. Treatment of SCZD hiPSC neurons with loxapine ameliorated fundamental cellular and molecular elements of the SCZD phenotype.	2011	(45)
Familial dysautonomia (FD)	Neurology	This study demonstrated tissue-specific mis-splicing of IKBKAP in purified FD-iPSC-derived lineages. FD-iPSCs were also used to validate candidate drugs' ability to reverse aberrant splicing and ameliorate neuronal differentiation and migration.	2009	(46)

7. Ethical, legal, and social issues in using iPSCs for therapy

Over the years, many guidelines have been put forward to restrict unethical research and therapy with human subjects, such as the Nuremberg Code (1947), the Declaration of Helsinki, and the Belmont Report (1978). Both guidelines are widely regarded as cornerstones of human research ethics today. These guidelines still apply today, but things have changed, and new possibilities have emerged. Technology advancements impact the current ethics of today's rapidly changing society, so they need to be revisited and revised. Nonetheless, recognizing and adhering to ethical rules on a global scale is/will be a significant challenge, not only in individual countries. Due to technological advances in biomedicine and the emergence of new fields such as stem cell research and genome editing, these new technologies require new regulations to enable them to be applied in these broad fields, specifically regenerative medicine (47). In contrast to drug therapy, cell therapy is a live component with complex functions that cannot be regulated. Therefore, stem cells are used in cell replacement therapies under separate rules and conditions. It is currently recommended that patients receive cell, tissue, and stem-cell products from the US Food and Drug Administration (FDA) (47) and the European Medicines Agency (EMA) guidelines. Experts from around the world have also been involved in developing or updating specific policies for the use of stem cells in cell therapy by specialist associations. Most of the differences between these guidelines are minor. In their opinion, the most important ethical, legal, and social considerations related to cell therapy include (i) manufacturing conditions and characterization of clinical-grade cells, (ii) genetic material and confidential personal information, (iii) informed consent, (iv) genetic manipulation of the cells, and (v) intellectual property and patents (47).

8. Future prospects

Several methods have been identified so far; however, more research is still needed to identify the best method. Certainly, most of the efforts have been focused on faster, safer, and more efficient processes. Forced expression of specific genes is a method that has been studied more than others. Most research efforts have focused on non-recombinant mechanisms and optimization of gene composition required for producing induced pluripotent stem cells. Although recombinant protein is safer, the results obtained so far are not encouraging; this inefficient method works at a low speed. Though it has been established that the activity of synthetic molecules increases the efficiency, chemists and biologists need to produce or identify a group of molecules with a low molecular weight that can be used as transcription factors and replace protein extracts, work together to produce induced pluripotent stem cells without using genes. The use of miRNA is still a promising proposition. When a more accurate understanding of this type of RNA and its role in the dedifferentiation process is obtained, the problem of the non-integrative method will be solved using this technique. Efficiency and problems related to the non-integrative method are the main problems. The properties of induced pluripotent stem cells, such as epigenetic rearrangement, stability, genomic transcription, and integration, should not be underestimated, and their tumorigenic potential should be analyzed. Consequently, it can be said that induced pluripotent stem cells simulate embryonic stem cells and can be used as their substitute for use in cell therapy.

9. Conclusions

In the pharmaceutical industry and clinical practice, iPSCs have opened up new opportunities for stem cell research. It must be considered, however, that reprogramming technology has its ethical-social issues. It is imperative to adopt laws and standards for iPSC production/application to ensure ethical integrity while removing unnecessary barriers to the use of iPSCs in research and therapy. The cell donor and recipient must both provide informed consent. Because donated cells contain private information in the form of DNA, patients' and donors' privacy must be protected. During the process of cell

donation, donors should be informed of the time during which they may have control over their cells.

Ethical Considerations
All ethical principles are observed.

Authors' contributions
All three authors were involved in the design and formulation of the argument.

Funding
This article is written at personal expense.

Acknowledgments
No need.

Conflict of interest
There is no conflict of interest.

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