Invited Special Paper

INTERNATIONAL JOURNAL OF SURGERY

OPEN

Unlocking the therapeutic potential: odyssey of induced pluripotent stem cells in precision cell therapies

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Abstract

This review explores the application of induced pluripotent stem cells (iPSCs) in regenerative medicine. The therapeutic significance of iPSC-derived cell therapy within regenerative medicine, emphasizes their reprogramming process and crucial role in cellular differentiation while setting the purpose and scope for the comprehensive exploration of iPSC-derived cell therapy. The subsequent sections intricately examine iPSC-derived cell therapy, unraveling the diverse derivatives of iPSCs and striking a delicate balance between advantages and limitations in therapeutic applications. Mechanisms of action, revealing how iPSC-derived cells seamlessly integrate into tissues, induce regeneration, and contribute to disease modeling and drug screening advancements is discussed. The analysis extends to clinical trials, shedding light on outcomes, safety considerations, and ethical dimensions. Challenges and concerns, including the risk of tumorigenesis and scalability issues, are explored. The focus extends to disease-specific applications, showcasing iPSC-derived cell therapy as a promising avenue for various medical conditions, supported by illustrative case studies. Future directions and research needs are outlined, identifying areas for further exploration, safety considerations and potential enhancements that will shape the future landscape of iPSC-derived therapies. In conclusion, this review provides a significant understanding of iPSC-derived cell therapy's status that contemplates the implications for regenerative medicine and personalized treatment using iPSCs, offering a comprehensive perspective on the evolving field within the confines of a dynamic and promising scientific frontier.

Keywords: epigenetic analysis, iPSC-derived cell therapy, lentivirus, retrovirus, diabetes, CVS disorders, Alzheimer etc.

Introduction

Stem cells are recognized for their pluripotent nature, which means they can transform into various cell types and have the ability to continuously regenerate themselves. In mammals, there are two types of pluripotent stem cells: embryonic stem cells (ESCs), which are derived from the inner cell mass of blastocysts, and embryonic germ cells (EGCs), which are obtained from embryos after implantation. These stem cells have the potential to develop into various organs and tissues[1,2]. After the isolation of human embryonic stem cells by Thompson in 1998, the creation of human-induced pluripotent stem cells (hiPSCs) was first achieved by Takahashi and Yamanaka in 2007. Human pluripotent stem cells (hPSCs) have shown significant potential for regenerative medicine[3,4]. In 2006, Shinya Yamanaka and Kazutoshi Takahashi successfully generated mouse-induced pluripotent stem cells (iPSCs) using an alternative reprogramming technique. They utilized a retrovirus to transfer four

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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International Journal of Surgery (2024) 110:6432–6455

Received 8 February 2024; Accepted 17 June 2024

Published online 4 July 2024

http://dx.doi.org/10.1097/JS9.0000000000001892

reprogramming transcription factors into a somatic cell, specifically a mouse fibroblast. These factors were Oct3/4 (Octamer-binding transcription factor-3/4), Sox2 (Sex-determining region Y-box 2), Klf4 (Kruppel Like Factor 4), and c-Myc (avian myelocytomatosis viral oncogene homolog)[4], and c-Myc, which were known as the "OSKM factors"[5]. Pluripotent stem cells, or PSCs, can differentiate into cells from all three germ layers and multiply endlessly. Because of these two characteristics, PSCs are desirable sources of cell treatments for various injuries and illnesses[6]. The term "induced" pluripotent stem cells (iPSCs) denotes the capability to transiently introduce specific sets of transcription factors into somatic cells, even though these pluripotent cells may originate from embryos[7]. A range of scientific domains, such as discovering new drugs, toxicological study, and disease simulation, have made use of hiPSCs and their derived compounds as they offer a human-relevant cell source[8]. hiPSCs were promptly utilized to establish models representing human diseases in a laboratory setting and for pharmaceutical screening to check for potential toxicities and effectiveness as soon as the technology was developed. The use of human iPSCs is becoming more popular due to the increasing interest in phenotypic screening and the advantages they offer over traditional cellular screens. These benefits include their human origin, ease of use, ability to be expanded, capacity to generate almost any type of cell, lack of ethical concerns associated with human ESCs, and potential for developing personalized therapeutics using patient-specific iPSCs[2]. The generation of iPSCs has been accomplished using various somatic cell origins, including dermal fibroblasts, pancreatic beta cells, neural stem cells, mature B lymphocytes, liver cells, keratinocytes, and cord blood cells[9]. The use of iPSCs for disease modeling has generated significant excitement, offering unique potential in high-throughput drug discovery systems and safety pharmacology. This potential is particularly evident when combined with three-dimensional multicellular organoids such as personalized organ-on-chip models, gene/base editing, AI, and high-throughput methodologies in the "omics" field[10]. Perhaps the true potential of iPSCs is in their capacity to facilitate the creation of patient-specific or autologous stem cell-based treatments. With the capability to provide enduring engraftment without the need for immune suppression, autologous cell-based treatments could offer patients safer therapeutic options. Three autologous iPSC-derived cell transplants have taken place in humans since the discovery of iPSCs, with the first transplant being carried out in 2014. Though there is no immunosuppression, the participant's trial which plays a significant role in the development of regenerative medicines[11]. The purpose of this review is to evaluate the current status of therapies using iPSCs and explore their potential applications in various regenerative disorders. This comprehensive assessment focuses on the use of iPSCs therapeutic utility, and it also includes a detailed analysis of the role of technology in regenerative medicines. The primary objective is to evaluate the feasibility, safety, and effectiveness of iPSC-derived cell therapies in both preclinical and clinical settings. This involves examining advancements in differentiation protocols, cell reprogramming techniques, and optimizing cell culture systems to ensure the production of high-quality and functional iPSC-derived cells. The review aims to introduce iPSCs and their reprogramming process, outline the various cell types that can be derived from iPSCs, evaluate the advantages and disadvantages of iPSC-derived cell therapy, and explore how iPSC-derived cells integrate into tissues, promote regeneration, and contribute to disease modeling. Furthermore, the review analyzes preclinical studies and clinical trials, addressing challenges, concerns, and disease-specific applications. Lastly, the objective is to discuss future directions, research needs, and the implications of using iPSCs in personalized therapy and regenerative medicine.

HIGHLIGHTS

- Introduction to induced pluripotent stem cells (iPSCs) in regenerative medicine: The review begins by highlighting the therapeutic importance of iPSC (induced pluripotent stem cell)-derived cell therapy in regenerative medicine, focusing on their reprogramming process and critical role in cellular differentiation.
- In-depth examination of iPSC-derived cell therapy: It delves into the various derivatives of iPSCs, discussing both their advantages and limitations for therapeutic use, balancing the potential benefits against the challenges.
- Mechanisms of action and integration: The ways in which iPSC-derived cells integrate into tissues and induce regeneration are closely examined, along with their contributions to disease modeling and drug screening advancements.
- Review of preclinical studies: The review meticulously analyzes preclinical studies, covering experimental methodologies, controls, and key findings to bridge the gap between laboratory research and clinical application potential.
- Clinical trials and outcomes: It discusses the results of clinical trials, focusing on patient outcomes, safety considerations, and the ethical aspects of using iPSC-derived therapies.
- Challenges and regulatory concerns: The review addresses significant challenges like tumorigenesis risk and scalability, and explores the regulatory and ethical considerations in the field.
- Disease-specific applications: It highlights the application of iPSC-derived cell therapy in treating various medical conditions, supported by case studies to showcase their potential and effectiveness.
- Future directions and research needs: The review outlines areas for further research and development, including potential enhancements and the regulatory pathways crucial for advancing iPSC-derived therapies.
- Conclusion and implications: Finally, the review concludes by offering a comprehensive perspective on the current status, promises, and limitations of iPSC-derived cell therapy, contemplating its implications for the future of regenerative medicine and personalized therapy.

iPSC-derived cell therapy and applications

Stem cells possess the potential to transform medicine by repairing damaged tissues and providing cures for various diseases. Pluripotent stem cells having distinct characteristics of pluripotency and self-renewal, obtained from the internal cell mass of a blastocyst, and these are commonly known as ESCs[12] (Fig. 1). However, ESCs are ethically controversial as they are

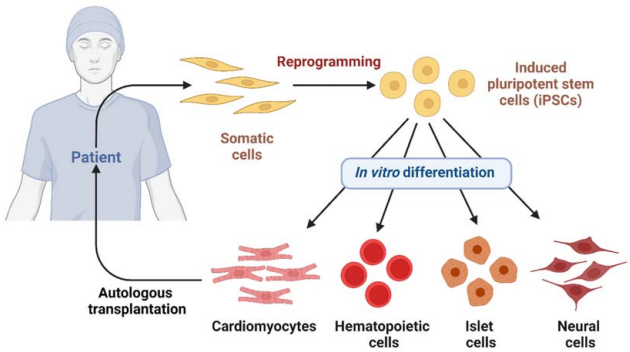

Figure 1. iPSC derived stem cell therapy. iPSC, induced pluripotent stem cell.

derived from embryos[13]. Nevertheless, two significant challenges have impeded their widespread use: ethical concerns stemming from destroying human embryos to obtain ESCs and encountering immunological rejection when ESC-derived cells are transplanted into non-donor recipients[14]. iPSCs provide an alternative reservoir of pluripotent stem cells that do not originate from embryos. They are mature cells that undergo genetic reprogramming to attain a state resembling ESCs. This transformation is accomplished by inducing the expression of particular gene transcription factors crucial for preserving ESC properties[15]. Like ESCs, iPSCs can proliferate indefinitely *in vitro* while retaining their self-renewal capacity. Additionally, they can differentiate into a wide range of cell types originating from all three primary germ layers (ectoderm, mesoderm, and endoderm), including germ cells responsible for generating gametes[14]. Somatic cells, differentiated cells with distinctive characteristics, are the most readily available in the body. The concept of transforming a specialized somatic cell into a pluripotent stem cell or potentially a totipotent embryo was put forth years ago. However, it only became a reality with the birth of the cloned sheep "Dolly" in mammals, as documented by Wilmut and colleagues in 1997[16]. iPSCs have tremendous potential in therapeutic applications due to their unique characteristics. Some of the applications are discussed as follows: Disease modeling is one of the main ways that iPSCs are revolutionizing medical science. Researchers can produce in-vitro models that accurately replicate the clinical characteristics of a disease by isolating stem cells (iPSCs) from individuals suffering from genetic abnormalities or other illnesses[17]. By differentiating these patient-specific iPSCs into the pertinent cell types impacted by the illness, scientists can learn more about the disease's causes, find new therapeutic targets, and evaluate the safety and effectiveness of possible medications. Additionally, the use of iPSC-based illness models opens the door to personalized medicine, in which patients' unique genetic makeup can be considered when designing a treatment plan, perhaps leading to better therapeutic outcomes[18]. Patient-derived iPSCs have been utilized to create various cardiovascular models, including long QT syndrome (LQTS), hypertrophic cardiomyopathy, Leopard syndrome, and arrhythmogenic right ventricular cardiomyopathy/dysplasia[19]. It is reported that using iPSC cardiomyocytes from LQTS patients revealed that a mutation in KCNQ1 causes dysfunction in the potassium ion channel, resulting in a deficiency in the sarcolemma. This is supported by the fact that iPSC-derived cardiomyocytes from patients show that treatment with potassium channel enhancers causes the same shortening. By using a new heterozygous exon 7 deletion mutation of KCNQ1, hiPSC-CMs were produced from an LQT1 patient, accurately reproducing the LQT1 symptoms, likely related to haploinsufficiency and trafficking impairment of KCNQ1/Kv7.1. The small chemical ML277 may be useful as a treatment for LQT1 patients, as it restored IK function in hiPSC-CMs[20]. The cellular characteristics linked to the processing of amyloid precursor proteins, such as those associated with amyloid precursor protein (APP), presenilin, and SORL1 mutation, are also evident in IPSC-derived neurons. Dysfunction of these proteins may lead to abnormalities in γ-secretase activity, endoplasmic reticulum function, and oxidative stress, resulting in tau protein hyperphosphorylation and accumulation of amyloid β peptide (Aβ)[21]. Docosahexaenoic acid therapy was found to be able to reduce the related stress responses in a study using iPSC-derived neurons from Alzheimer's patients. Another study showed that using a drug screening platform, certain anti-Aβ compounds could inhibit Aβ plaque deposition in patient-derived cortical neurons. iPSCs have enormous potential for drug research and discovery in addition to disease models. Conventional drug screening techniques frequently depend on immortalized cell lines or animal models, which might not be a realistic representation of human physiology and disease processes[8]. Indeed, iPSCs offer a more accurate platform for drug testing. Using patient-derived iPSCs, researchers can evaluate potential medications in a human setting, leading to the identification of safer and more effective treatments. iPSC-based drug screening can also provide more predictive preclinical data, ultimately reducing the failure rates of drug candidates in clinical trials and expediting the delivery of innovative medicines to patients. Furthermore, iPSCs show promise in regenerative medicine through cell replacement therapy. For instance, iPSCs could potentially be used to replace damaged or malfunctioning cells in various tissues and organs, such as neurons to treat neurodegenerative diseases like Parkinson's or Alzheimer's, thus restoring brain function[22]. Individuals with heart disease may benefit from the use of iPSC-derived cardiomyocytes for cardiac repair. This could potentially reverse the damage caused by myocardial infarction or other cardiac disorders. While obstacles such as immune rejection and tumorigenicity need to be addressed before iPSC-based cell therapies are widely used, current research in this field shows promise for the development of safe and effective treatments for a wide range of diseases[23]. Induced pluripotent stem cells (iPSCs) have shown potential as a cutting-edge method for treating autoimmune disorders and cancer through immunotherapy. It is possible to genetically modify iPSCs to produce therapeutic proteins or antigens. This modification enables the production of immune cells with specific functions, such as destroying cancer cells or adjusting the immune system in cases of autoimmune diseases. For example, chimeric antigen receptor-expressing T cells produced from iPSCs (iPSC-derived T cells) have demonstrated impressive efficacy in treating specific forms of leukemia and lymphoma, resulting in long-lasting remissions for several patients[24]. Similarly, the potential for long-term disease management with minimal side effects exists when utilizing iPSC-derived regulatory T cells to reduce abnormal immune responses in conditions such as multiple sclerosis or rheumatoid arthritis[25]. iPSCs can be produced from somatic cells using diverse techniques, such as somatic cell nuclear transfer (SCNT),

cell fusion, and introducing OSKM transcription factors and small molecules[12]. Several cell types are outlined as follows.

Somatic cell nuclear transfer (SCNT)

SCNT is how adult somatic cells can be reprogrammed through fusion with a mature oocyte. Blastocysts generated via SCNT demonstrated competence by successfully generating live animals and extracting ESCs from their inner cell mass. This illustrates the ability of somatic nuclei to undergo reprogramming to achieve a pluripotent state facilitated by factors present in the oocyte cytoplasm. As a result, these reprogrammed nuclei can guide embryonic development to its full term[26]. Nonetheless, most cloned animals display mild to severe phenotype and gene-expression abnormalities, indicating that SCNT leads to defective epigenetic reprogramming[27].

Cell fusion

Another demonstrated approach that has been shown to reprogram somatic cells into pluripotent cells involves fusing somatic cells with pluripotent cells. Nevertheless, the effectiveness of this method is constrained since the resulting cells become tetraploid. The process involves intricate combinations of both identified and unidentified factors. Initiating reprogramming from oocytes or pluripotent cells complicates mechanistic studies[28,29].

OSKM transcription factors and small molecules

To overcome the barriers in the case of SCNT and cell fusion, a new strategy transforming mammalian somatic cells into iPSCs through the introduction of pluripotent transcription factors (TFs) such as Oct4, Sox2, Klf4, and c-Myc (or Nanog and Lin28 as alternatives to Klf4 and c-Myc) is employed. The process entails the epigenetic control of genes associated with typical cell development. In the OSKM combination, Oct3/4, Sox2, and Klf4 positively regulate genes to maintain ESC pluripotency. Simultaneously, they suppress the expression of genes that encourage differentiation. While c-Myc is not essential for reprogramming, its inclusion enhances efficiency by influencing cell proliferation rather than pluripotency[30]. Over the past ten years, multiple research teams have endeavored to address these challenges by employing various transcription factors to reprogram somatic cells, including OSNL, OSML, OSK, and others[31]. TFs specific to cell types preserve the identity of cells by attaching to distinct DNA sequences and interacting with co-regulatory factors. Externally introduced TFs in iPSC generation collaborate to remodel chromatin, activating pluripotency genes and suppressing differentiation genes. While iPSC reprogramming is technically less complex than SCNT and cell fusion, it triggers a dynamic and undefined reprogramming process. As a result, it exhibits lower efficiency and a slower pace when contrasted with SCNT and cell fusion. Small molecules influencing epigenetic processes can improve the efficiency of reprogramming by altering DNA methylation, as exemplified by 5-aza-cytidine and RG108, histone acetylation affected by sodium butyrate and trichostatin A, or histone methylation, as demonstrated with Neplanocin A. The small molecule approach indirectly triggers iPSC reprogramming by altering non-pluripotency-specific elements in somatic cells. Its primary mode of action is to inhibit the expression of genes linked to cell development and differentiation. Substituting various small molecules, such as valproic acid, CHiR99021, sodium butyrate, vitamin C, Parnate, 5-Azacytidine, and RG108, for components within the core pluripotent network can significantly increase the efficiency of the reprogramming process[32]. It takes approximately 2 weeks of factor expression to induce pluripotency in human cells, along with the time needed for the repetitive transfections required to generate iPSCs[33]. (Fig. 2)

Trans-differentiation and reverse differentiation of iPSC

The progress in stem cell biology has led to a better understanding of specific cell lineages, making it possible to generate many specialized cell types from stem cells. Additionally, researchers have identified distinct developmental stages governed by genetic and epigenetic regulatory networks. At the same time, an alternative method known as trans-differentiation is being investigated to directly convert one somatic cell type into another. This approach could involve using abundant adult cells such as dermal fibroblasts or adipocytes to produce other important therapeutically relevant cells like neurons, cardiomyocytes, or pancreatic beta cells[34]. Trans-differentiation is considered a safer option compared to reprogramming. Directly transforming differentiated cells into different cell types bypasses the need for achieving pluripotency.

Consequently, cells obtained through trans-differentiation do not inherently gain the capacity for uncontrolled self-renewal and proliferation, as seen in reprogramming. While this decreases the risk of cancer formation, there is still a possibility of carcinogenesis due to potential genetic and epigenetic alterations during the trans-differentiation process[35]. Recent advances in trans-differentiation present an intriguing avenue for modeling neurological diseases. This approach involves converting easily accessible cells such as fibroblasts or PBMCs into specific types of neural cells like neurons, astrocytes, and microglia. One significant advantage is the potential to use cells derived from patients, which may reflect age-related disease factors. This complements existing research that uses iPSCs, as iPSCs can lose disease characteristics during reprogramming. However, there are practical challenges to trans-differentiation. Firstly, there's a lack of robust protocols for efficiently generating specific neural cell types. Secondly, the conversion yield is often low. For example, the transformation of blood cells into neurons resulted in only around 3% conversion. In such cases, generating progenitor cells from PBMCs or fibroblasts might be more effective. These progenitors can be expanded and cryopreserved, creating a long-term resource for differentiated neural cells. Beyond trans-differentiation, neurological cell-based models face additional challenges. Achieving uniformity, maturity, and specificity of the generated cells remains difficult. For instance, trans-differentiation protocols may produce mixed neuronal populations expressing markers from both the central and peripheral nervous systems.

Moreover, the functionality of these cells may be limited, with studies showing restricted synaptic formation compared to neurons derived from iPSCs or neural precursors. In conclusion, trans-differentiation shows promise as a strategy for modeling neurological diseases using patient-specific cells. However, overcoming limitations in conversion efficiency, cell type specificity, and maturity is crucial for its widespread adoption[36]. According to the Cell Reversion Theory, a differentiated cell can transition to an iPSC state, potentially passing through multipotent cell (MC) states in response to changes in its conditions or

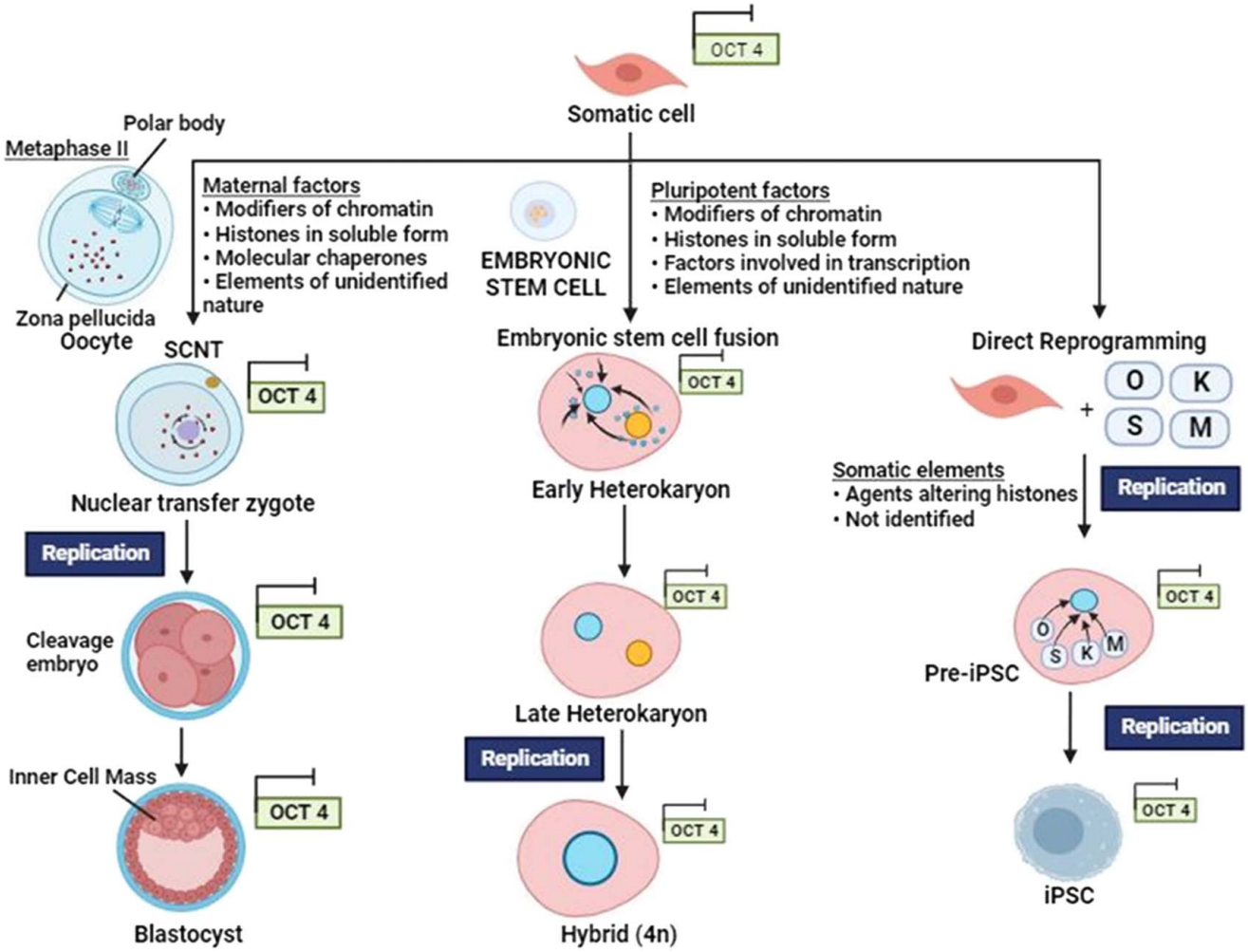

Figure 2. Approaches for reprogramming somatic nuclei. iPSC, induced pluripotent stem cell; SCNT, somatic cell nuclear transfer.

environment, such as chemical or mechanical stimuli. The resulting iPSC, displaying stem cell-like characteristics, may proliferate and differentiate uncontrollably, creating a stressful cellular environment similar to hypoxia, possibly leading to the formation of tumors. This theory contrasts with the Somatic Mutation Theory, where successive mutations induced by carcinogenic events drive the development of the tumor phenotype[37].

Characterization of iPSCs

During the reprogramming of somatic cells, it is essential to thoroughly characterize cell lines and evaluate colonies to ensure the establishment of reprogrammed iPSCs. Table 1 illustrates the methods of characterization of iPSC[38,39]. Since the supply of donated organs and tissues is limited, iPSCs offer a renewable source for fulfilling the demand for replacement cells and tissues upon differentiation into particular cell types. Table 2. represents applications and various types of organoids derived from iPSCs.

In contrast to embryonic stem cells, iPSCs are obtained from adult cells, avoiding usage restrictions. They have stable proliferation, provide an unlimited cell source, and can undergo differentiation into over 200 cell types in the body[40]. Their specific-to-the-patient characteristics enable the investigation of disease mechanisms and experiments about treating inherited conditions. Emphasizing safety and diminishing dependence on animal cells is crucial for broader human use. Additionally, the capability to produce iPSCs from large animals like humans enhances research capabilities and supports improvements in animal welfare[41]. iPSC shows great promise for clinical use, but there are significant challenges to address. These include the risk of tumor formation (teratoma) during transplantation, issues related to genetic alterations and reprogramming factors, potential immune rejection requiring immunosuppressants, and variations in laboratory results. Heterogeneity in genetics and gene expression further complicates iPSC applications[42]. Another significant drawback is the time needed to establish stable iPS cell lines. Addressing these challenges is essential for progressing the clinical potential of iPSC technology. Tables 3 represents the comprehensive information on clinical trials of iPSC.

Delivery methods for reprogramming methods

Reprogramming factors are introduced into somatic cells using a variety of techniques. These approaches can be categorized into two

Table 1

Characterization of iPSCs.

Morphology	Circular form, featuring a prominent nucleolus and minimal cytoplasm. Dense colonies with well-defined borders and highly active in mitosis because of their self-renewable characteristics.
Pluripotency markers	Display cell surface proteins like transcription factors Oct4, Nanog, Sox2, Tra-1-81, alkaline phosphatase, SSEA-4, etc.
Developmental potential	Embryoid generation: Assessed through immunocytochemistry techniques targeting pluripotent markers. Teratoma formation - Determined by immunohistochemistry method for differentiation markers
Genetic examination	Karyotyping (test to examine chromosomes in a sample of cells) Silencing of transgenes following the process of reprogramming.
Epigenetic analysis	Methylation of genes associated with specific cell lineages.

iPSC, induced pluripotent stem cell.

types: integrative systems, which entail non-integrative systems, and the integration of exogenous genetic material into the host genome, which avoids integrating genetic material into the host genome[43,44]. Figure 3 represents the classification of delivery methods.

Integrative delivery systems

Viral integrative vectors

Retrovirus: The OSKM transcription factors were initially introduced into mouse or human fibroblasts using retroviruses that were derived from Moloney murine leukemia virus (MMLV), such as pMXs, pLib12, or pMSCV. These retroviral vectors, having a cloning capacity of ~8 kb, facilitate the delivery of genes into the genomes of dividing cells. They are typically muted in immature cells like ESCs, a crucial element because successful reprogramming boosts the endogenous pluripotency gene network and reduces transgene expression to achieve a fully reprogrammed iPSC[45]. Retroviruses are effective in gene transfer as they allow extended transgene expression after genomic integration and exhibit a lack of immunogenicity[45]. They serve as a very effective and straightforward delivery method, with optimal performance observed when introduced into actively dividing somatic cells to integrate into the genome successfully. Administering regulators in cells that are not actively dividing or

Table 2

Applications and various types of organoids derived from induced pluripotent stem cells.

Range of cell type developed by IPSCs	Induction process	Function	References
Cardiomyocytes	The four transcription factors Oct4, Sox2, Klf4, and c-Myc were used to create the human-induced pluripotent stem cell line.	Combining human iPSC-derived cardiomyocytes (hiPSC-CMs) and the omentum flap might assist in developing new, vascular-rich heart muscle *in vivo*.	[33]
Retinal pigment epithelial cells	Transduction using Oct3/4, Sox2, Klf4 and c-Myc	Treatment of retinal degenerative disease	[34]
Dopaminergic neurons	Human iPSC line was generated from the peripheral blood cells of a healthy volunteer who was homozygous for the human leukocyte antigen (HLA).	Treatment of Parkinson's disease	[35]
Natural killer cells	iPSCs derived using blood cells from the umbilical cord	Immunotherapy for ovarian cancer	[36]
Neural progenitor cells	The 414C2 and 201B7 lines of human-induced pluripotent stem cells (iPSCs) were cultured using SNL murine fibroblast feeder cells treated with mitomycin C, utilizing standard hESC medium enriched with specific supplements and growth factors, within an environment containing 3% CO_2.	Complete treatments for spinal cord injury	[37]
T lymphocytes	Induced pluripotent stem cell clones (T-iPSCs) were produced through the transduction of peripheral blood T lymphocytes (PBL) obtained from a healthy volunteer. Two retroviral vectors were utilized, each carrying two reprogramming factors—KLF4, SOX2, OCT4, and C-MYC.	Cancer immunotherapy	[38]
β cells	Fibroblast cells were transformed into induced pluripotent stem cells (iPSCs) via the introduction of hSOX2, hOCT3/4, hKLF4, and hC-MYC genes using a lentiviral system.	Management of diabetes mellitus, encompassing both type-1 and type-2 conditions	[39]
Alveolar epithelial cells	iPSC-AECII underwent transduction with lentiviral vectors (LV) carrying human telomerase reverse transcriptase (hTERT) and polycomb complex protein BMI-1 (hBmi1).	Acute lung injury and severe respiratory distress syndrome	[40]
Endothelial cells	iPSCs were cultured conventionally, embryoid bodies (EBs) were employed for differentiation into endothelial cells (ECs), adipogenic, osteogenic, and chondrogenic lineages, and the resulting endothelial cells were subjected to transduction using lentiviral vectors (LVs) regulated by endothelial-specific promoters TIE-2, VEC, and FLK1.	Vascular tissue formation	[41]
Mesenchymal stem cell-derived chondrocytes	The iPSC line was derived from Normal Human Epidermal Keratinocytes (NHEK) and was created using the Sendai virus reprogramming system. This system employed virus particles to transport a polycistronic combination of KLF4–OCT3/4–SOX2, CMYC, and KLF4.	Repair cartilage defects in osteoarthritis	[42]

Table 3

Comprehensive information on clinical trials of iPSC.

Clinical trial ID	Title of study	Indication	Country	Year
NCT06145711	A Clinical Trial of Parkinson's Disease Treatment by Human-induced Pluripotent Stem Cells (hiPSCs) Derived Dopaminergic Neural Precursor Cells	Parkinson	China	2023
NCT06147505	Clinical Study of Evaluating the Safety and Initial Efficacy of XS005 Cell Injection Combined With Stupp Regimen for Adjuvant Chemotherapy in Subjects With Primary Glioblastoma	Glioblastoma	China	2023
ACTRN12623000202662	A First-in-Human (FIH), Open-Label, Phase la Dose Escalation Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of SON-DP in Subjects with Advanced/ Metastatic Solid Tumours that have relapsed or are refractory/ intolerant to standard of care therapies	Solid tumors	Australia	2023
NCT05647213	Safety and Feasibility of Autologous Induced Pluripotent Stem Cells of Cardiac Lineage in Subjects With Congenital Heart Disease	Congenital Heart failure	United State	2022
NCT05616338	Modeling Bronchial Epithelium in Severe Asthma With Human Induced Pluripotent Stem Cells (iPSC)	Asthma	France	2022
ISRCTN12295348	Modelling and rescue of inherited retinal diseases using induced pluripotent stem cell (iPSC)-derived retinal cells and organoids	Retinal disorder	United Kingdom	2022
NCT05647213	Safety and Feasibility of Autologous Induced Pluripotent Stem Cells of Cardiac Lineage in Subjects With Congenital Heart Disease	Congentional Heart failure	United States	2022
DRKS00025472	Generation of induced human pluripotent stem cells (iPSC) from patients with uro-genitary diseases and corresponding control persons for the investigation of the uro-genitary tract	Uro-genitary infection	Germany	2021
JPRN-jRCT2033210163	A phase I/II study of human induced Pluripotent Stem (iPS) cell-derived cardiomyocyte spheroids in patients with severe heart failure, secondary to ischemic heart disease, undergoing coronary artery bypass grafting - LAPiS Study	Congentional Heart failure	Japan	2021
IRCT20200429047241N1	Personalized Immunology of Patients with Advanced Breast Cancer Using induced Pluripotent Stem Cell-Derived Natural Killer cells	Breast cancer	Iran	2020
JPRN-jRCTa031190228	Regenerative medicine for spinal cord injury at subacute stage using human induced pluripotent stem cell-derived neural stem/progenitor cells	Spinal cord injury	Japan	2020
ACTRN12620000612910	A pilot, open-label, randomised controlled clinical trial to investigate early efficacy of CYP-001 in adults admitted to intensive care with respiratory failure (due to COVID-19 or another underlying condition).	COVID-19	Australia	2019

have slow division rates proves challenging in retroviral-mediated methods. This challenge may result in genes integrating haphazardly, leading to potential issues like chromosomal abnormalities or teratoma formation. The effectiveness of retroviral transduction falls within the range of 0.1–1%. The low efficiency in reprogramming is likely due to the limited infection

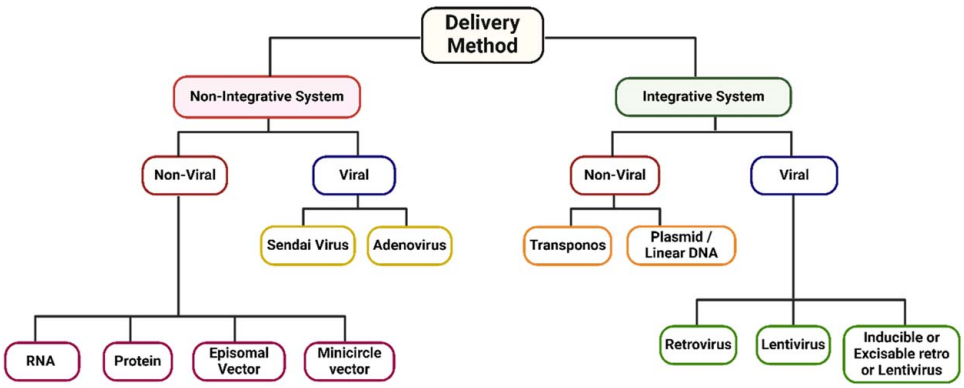

Figure 3. Delivery methods of reprogramming factors.

efficiency of cells by viruses. Implementing "secondary reprogramming systems" to uniformly activate reprogramming factors has elevated efficiency to 1–5%, suggesting that viral infection plays a role in reprogramming efficiency. Another potential factor is the activation of endogenous genes through insertional mutagenesis[27,46].

Lentivirus: Primarily sourced from HIV, they demonstrate slightly greater cloning capacity (8–10 kb) and typically boast increased effectiveness of infection compared to retroviruses based on MMLV. Additionally, they permit infection in both proliferating and non-proliferating cells. Lentiviral integration sites are commonly distributed throughout the transcriptional unit, while gamma-retroviruses tend to integrate close to transcriptional start sites. The regulation of its manifestation is achieved by administering the doxycycline medication, enabling the targeted selection of reprogrammed cell lines. Using lentiviral vectors has clear advantages like efficient and long-lasting transgene expression with limited immunogenic response. However, drawbacks include limited insertion size, storage challenges, and a short half-life[47].

Inducible or excisable retro or lentivirus: The combination of both the excisable (Cre/loxP) vector system and the inducible (tetracycline/doxycycline-inducible) vector system has improved the regulation of transgene expression. This helps to minimize the impact of ineffective silencing and the reactivation of transgenes. Although their preparation is more complex and time-intensive compared to MMLV-derived retroviruses, their significant advantage is their ability to serve as inducible systems[35].

Non-viral integrative vectors

Multiple factors impact the effectiveness of gene transfer. Present obstacles encompass insertional mutagenesis using viral vectors, inappropriate transgene expression, and immune reactions targeting the vector, genetically modified cells, or the transgene product. These challenges may result in inconsistent transgene expression, elimination of modified cells, acute systemic toxicity, and potentially the development of transformed cell growth and oncogenesis. Therefore, creating secure and effective alternatives utilizing non-viral vectors is necessary, as they exhibit lower immunogenicity than viral vectors[48].

Transposons: Transposable elements (TEs) are characterized as DNA sequences capable of relocating within the genome from one position to another[49].

PiggyBac (PB) transposon: The PiggyBac (PB), a transposon carrying a transposase enzyme, facilitates gene transfer. The transposon effectively integrates by co-transfecting a donor plasmid with a helper plasmid expressing the enzyme. After reprogramming, the enzyme can accurately remove transgenes without causing genetic harm, thereby avoiding the potential for insertional mutagenesis. Disadvantages of using PB transposons include time-consuming processes and the potential inefficiency of excision[50].

Sleeping beauty: This non-viral vector merges the strengths of viruses and naked DNA. It consists of a transposon with a gene-expression unit and a provider of transposase enzyme. Transposing the expression unit into the genome enables sustained transcription of a transgene. The sleeping beauty (SB) transposon has addressed several drawbacks of the PB transposon. SB has lower integration than PB, and no elements like SB exist in the human genome[50].

Non-integrative delivery systems

Employing viral techniques to incorporate reprogramming factors into iPSCs offers efficiency but comes with risks, restricting its clinical applications. The persistent presence of Yamanaka factors, such as c-Myc, results in genetic and epigenetic mutations, transcriptional abnormalities, and gene network instability. In response to safety concerns, developing new protocols aims to derive iPSCs without integration, concurrently addressing problems related to efficiency challenges observed in earlier methods[51].

Viral non-integrative delivery systems

Adenovirus

Employing Adenovirus to deliver reprogramming factors demonstrates a specific level of efficacy, enabling the production of relatively safe iPSCs without integration. Stadtfeld and team achieved the creation of the initial integration-free iPSCs from adult mouse hepatocytes using non-integrating adenovirus. Subsequently, Zhou & Freed (2009) employed similar adenoviral vectors to generate transgene-free iPSCs from human fibroblasts[5]. However, the effectiveness of this method in reprogramming is minimal, ranging from 0.001 to 0.0001% in mice to 0.0002% in human cells. To make adenovirus practical for reprogramming, substantial efforts are needed to optimize expression and enhance reprogramming efficiencies[52].

Sendai virus

The Sendai virus, an RNA virus, infects various cell types without entering the host cell nucleus. RNA virus vectors, like those derived from Sendai, are deemed suitable for transporting Yamanaka factors due to their low risk of genomic insertion. These vectors are commonly employed for reprogramming neonatal and adult fibroblasts, as well as blood cells. Moreover, the virus in the cytoplasm during replication can be removed from host cells through successive passages. Reprogramming cells with the Sendai virus take around 25 days, with efficiencies of 0.1% for blood cells and 1% for fibroblasts. However, a constraint is that it takes approximately ten passages for the virus to be eliminated from recently reprogrammed iPSCs, and cells might need to be cultured at an elevated temperature (39°C) for comprehensive virus eradication[52,53].

Non-viral non-integrative delivery systems

Warren and colleagues established a system that effectively transforms diverse human somatic cells into iPSCs through the direct administration of synthetic mRNAs. This approach attains notably greater efficiency than other non-integrative systems, successfully converting 2% of neonatal fibroblasts into iPSCs within a mere 17 days, eliminating the need for plasmid or viral vectors[45]. Among non-integrative delivery systems, this approach demonstrates the highest efficiency in reprogramming. Because RNA has a short half-life, the reprogramming process requires frequent transfections to be maintained. Furthermore, RNA-based techniques have a reputation for being very immunogenic. To boost the efficiency of this method, mRNA delivery is paired with hypoxic culture conditions, doubling reprogramming effectiveness. Yet, direct cell reprogramming using mRNA carries

risks. The frequent administrations needed for high protein expression can activate c-Myc, posing a significant risk for tumor development[54].

Protein

Reprogramming factors can be delivered as proteins to circumvent the introduction of exogenous genetic material. Multiple studies indicate that proteins, when combined with transduction peptides such as HIV transactivator of transcription (Tat) and poly-arginine, can be delivered directly into cells *in vitro* and *in vivo*. Zhou and colleagues applied this approach by generating recombinant OSKM proteins fused with a poly-arginine transduction domain. Reprogramming with recombinant proteins poses challenges and requires various enhancements. Ensuring a consistent protein synthesis is difficult, demanding specific skills, making the technique ineffective for many laboratories[55].

Episomal vectors

Episomal vectors offer an alternative to integrative-defective viruses. Episomes, which are extrachromosomal DNAs capable of independent replication within a cell, allow direct and temporary transfection of reprogramming factors into somatic cells using episomal vectors in the form of plasmids. These techniques are appealing as they are straightforward to execute in a conventional laboratory with expertise in molecular biology, avoiding the labor-intensive and time-consuming generation of viral particles[56].

Minicircle vectors

Minicircle vectors are compact vectors comprising solely the eukaryotic promoter and the cDNA(s) intended for expression. For instance, a minicircle vector expressing Lin28, GFP, Nanog, Sox2, and Oct4 in human adipose stromal cells successfully reprogrammed 0.005% of the cells within ~28 days. Genes encoded by minicircles exhibit high efficiency in proliferating and non-proliferating cells, increasing desired protein expression levels. This is due to their reduced susceptibility to inactivation and silencing by cellular mechanisms that typically act on foreign nucleic acids. The transfected cells are cultured on feeder layers (various cell types, such as fibroblasts or keratinocytes, have been shown to serve as feeder cells) in suitable media conditions. Following the expression of reprogramming factors, iPSCs are produced. The cultured iPSC colonies can be identified using various morphological and physicochemical techniques[57].

Preclinical trials

Somatic cell reprogramming was first demonstrated with mouse and human cells. The finding that rat and non-human primate cells can be reprogrammed using the same transcription factors suggests that the mechanisms underlying pluripotency induction are conserved across the family Mammalia. Additionally, iPSCs have been derived from dogs, rabbits, various non-human primate species, and, more recently, domestic animals like horses, pigs, cows, sheep, and goats[58]. In July 2007, two independent groups reported generating mouse-iPSCs with enhanced germline competence by selecting for Nanog or Oct4 expression. Oct4 and Nanog are essential for managing undifferentiated ESCs. By reprogramming fibroblasts from transgenic mice via retroviral transduction of Oct4, Sox2, c-Myc, and Klf4, nanog-iPSCs were produced. When compared to Fbxo15-iPSCs, these iPSCs and Oct4-iPSCs showed advanced development traits. Their equivalency to mouse ESCs is debatable, though, and reactivating c-Myc enhanced the tumorigenic potential of chimeric mice injected with Nanog-selected iPSCs[4].

In-vitro and in-vivo experiments using iPSC-derived cells

So Gun Hong and colleagues focused on the generation of Rhesus iPSCs where dermal fibroblasts, BMSCs, or CD34 + cells were transduced with a lentiviral vector containing reprogramming factors (OCT4, SOX2, KLF4, and MYC). After transfer to specific culture conditions and selection, colonies with ESC-like morphology were obtained and characterized for pluripotency. Cre-mediated excision was employed to remove the reprogramming factors. Immunohistochemistry confirmed pluripotency markers, and teratoma assays in mice demonstrated their pluripotent nature. Furthermore, differentiation protocols towards mesodermal stromal-like cells and osteogenic lineages were established, and in-vivo transplantation studies were conducted to assess tissue grafting and bone formation. Histological analyses provided insights into the characteristics of tissue grafts. This comprehensive methodology generated Rhesus iPSCs and explored their potential for differentiation and transplantation *in vivo*, laying a solid foundation for further investigations[59]. Nigel and colleagues' study investigated the efficacy of combination therapy using CpG adjuvant and FVB strain iPSCs in combating murine breast cancer (DB7). The study established that a 4-week vaccination schedule with the CpG and iPSCs (C + I) combination produced the most robust immune response against DB7 tumor lysate regarding in-vitro T-cell responses and immunoglobulin G (IgG) binding. Following optimizing the vaccination schedule, 40 FVB mice were divided into four groups: PBS, CpG, iPSCs, and C + I. Subcutaneous injections of DB7 cancer cells were administered following four weekly vaccination sessions. The results showed that the C + I-treated mice exhibited regression of lesions at the injection site in 7 out of 10 cases, whereas other groups experienced tumor progression.

Further analysis of immune profiles in blood, spleen, and draining lymph nodes (dLNs) after 4 weeks post-tumor inoculation revealed promising results in the C + I group. Long-term survival studies showed that while most mice were sacrificed within two weeks due to tumor size exceeding 1 cm^3, two mice from the group treated with C + I survived for one year. In a prophylactic setting, iPSC vaccines prevent tumor growth in syngeneic murine breast cancer, mesothelioma, and melanoma models. As an adjuvant, the iPSC vaccine inhibited melanoma recurrence at the resection site and reduced metastatic tumor load, which was associated with fewer Th17 cells and increased CD11b + GR1hi myeloid cells. These mice displayed antibody titers against iPSCs and DB7 comparable to the initial stages of the experiment, and they successfully rejected reintroduced cancer cells (Fig. 4). The study also included control mice that were primed with endothelial cells derived from iPSCs, ruling out the possibility of cross-reactivity due to culturing conditions or endogenous murine leukemia viral antigens. Overall, the combination therapy of CpG and iPSCs demonstrated promising efficacy in inducing a robust immune response against murine

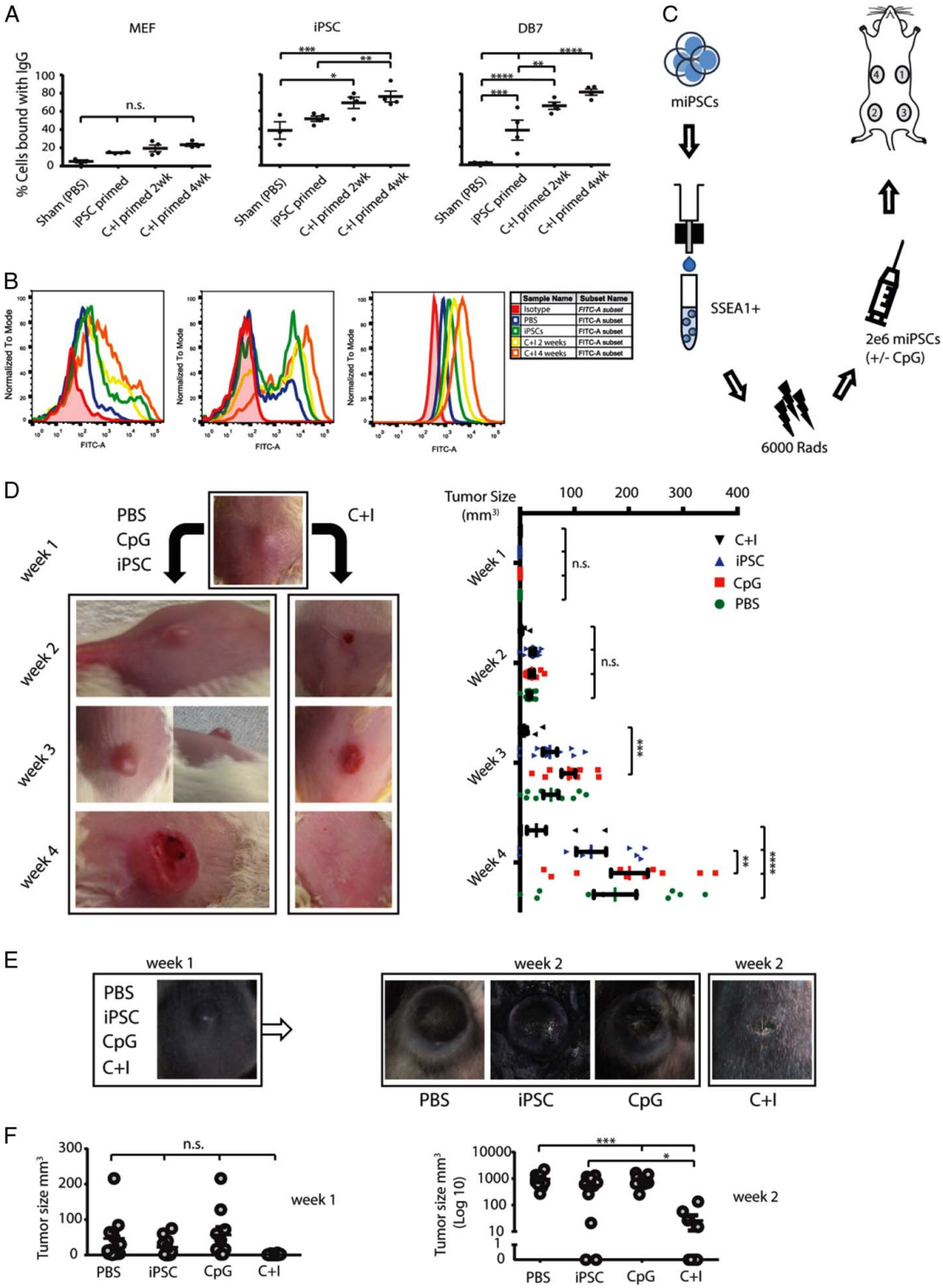

Figure 4. Evaluating the most effective vaccination timetable, succeeded by the successful preventive treatment of breast cancer and melanoma in mice (adapted with permission under CCBY 4.0 from[60]). iPSC, induced pluripotent stem cell.

breast cancer, leading to tumor regression and long-term survival in some cases[60,61].

(A) The optimal vaccination schedule was determined as C + I vaccination for four weeks, evaluated based on the percentage of IgG binding to DB7, with no significant increase in non-specific mouse embryonic feeder (MEF) binding ($n = 3$ control animals, $n = 4$ iPSC primed animals, n = 4 C + I primed 2-week animals, and $n = 4$ C + I primed 4-week animals, mean ± SEM, ANOVA with Tukey's multiple comparison test). (B) A representative FACS plot illustrates serum IgG binding of PBS 4-week, iPSC 4-

week, C + I 2-week, or C + I 4-week-vaccinated mice to embryonic fibroblasts (left panel), iPSCs (middle panel), and DB7 cancer cells (right panel). In the iPSC analysis, a partly differentiated cell culture served as a control sample for differentiated cells, indicated by IgG-positive and negative cells, confirming the specificity of IgG binding to the undifferentiated portion of the analyzed cells. C + I 4-week-vaccinated mice exhibited the highest IgG binding to DB7 breast cancer cells. The researchers collected 30 ml of midstream urine from two healthy Chinese volunteers, processed it through centrifugation, and cultured the urine-derived cells. After the initial incubation, the cells were passaged, and lentiviral transduction was performed using specific transcription factors (Sox2, Oct4, c-Myc, and Klf4) to reprogram them into iPSCs. The infection efficiency was monitored, and successfully reprogrammed iPSCs were further characterized using flow cytometry and immunofluorescence staining for pluripotency markers. To validate the pluripotent nature of the iPSCs, alkaline phosphatase staining and in-vitro differentiation into embryoid bodies (EBs) were performed. The researchers confirmed the pluripotency by assessing the expression of specific markers (Oct4, Nanog, SSEA-4, TRA-1-60) and through teratoma formation when the iPSCs were injected into mice. The study then focused on the cardiac differentiation of iPSCs, utilizing a medium free of serum and chemically defined with specific supplements. The differentiated cells were characterized using flow cytometry and immunohistochemistry for cardiac markers (Troponin T, α-actinin, MLC-2a, MLC-2v, HCN4). The researchers recorded action potentials of spontaneously beating clusters, identifying distinct atrial and ventricular cell types based on their electrophysiological properties The methodology demonstrated the feasibility of generating iPSCs from urine-derived cells and differentiating them into functional cardiomyocytes, suggesting potential applications in cardiac cell therapy. However, the study acknowledged concerns related to lentiviral integration and proposed exploring alternative vectors for reprogramming in future applications[62]. Schematic representation of iPSC generation from UC. SKOM refers to the four exogenous factors Sox2, Klf4, Oct4, and c-Myc (A) Embryoid body formation in suspension culture with spontaneously differentiation into the three germ layers (arrowheads in B-F): (B) smooth muscle actin (mesoderm), (C) nestin (ectoderm) and (D) alpha-fetoprotein (endoderm). In addition, human iPSC spontaneously differentiated into cardiomyocytes (troponin-I, E) and endothelial cells (vWF, F). Scale bars in A represent 500 μm and in B-F represent 50 μm .Human iPS cell-induced teratoma formation 5 weeks after subcutaneous injection in NOD/SCID mice. Staining with hematoxylin/eosin of the three germ layers (arrowheads) in the teratoma. (Fig. 5).

The study investigates the interaction between induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) and the glucocorticoid drug dexamethasone (Dex) in the context of immunomodulation. iPSC-MSCs are advantageous over bone marrow-derived MSCs due to various factors such as longer lifespan, more accessible in-vitro expansion, and multiple sources. The researchers successfully derived iPSC-MSCs from amniocyte-derived iPSCs, characterized their phenotype and functionality, and demonstrated their ability to inhibit lymphocyte proliferation and reduce inflammation in mouse models of allergic airway inflammation and contact hypersensitivity. The study specifically focuses on the potential interaction between iPSC-MSCs and Dex, a commonly used glucocorticoid in treating allergic diseases. The results show that the concurrent administration of iPSC-MSCs and Dex exhibits comparable immunoinhibitory effects *in vitro* and mouse inflammation models when compared to iPSC-MSCs alone. The iPSC-MSCs, even in the presence of Dex, effectively inhibit lymphocyte proliferation and reduce inflammation, suggesting that Dex does not compromise the immunomodulatory properties of iPSC-MSCs. The findings support the practicality of applying iPSC-MSCs alongside steroids in clinical scenarios and suggest that iPSC-MSCs, combined with even low doses of steroids, may be a viable approach for immunomodulation. However, the study acknowledges some limitations, such as the need for further investigation into the potentially disruptive effects of iPSC-MSCs in specific settings and the necessity of assessing differential subsets of inflammatory cells (Fig. 6). In conclusion, this preliminary evidence suggests a positive interaction between iPSC-MSCs and Dex, opening possibilities for combined therapeutic approaches in specific clinical scenarios[63].

Therapeutic applications of iPSC

Disease modeming with human iPSCs

The rationale behind using iPSCs to establish a model for a disease in a culture plate stems from these cells' extraordinary ability to continuously regenerate themselves and their capacity to transform into any specific cell type present in the human body. The primary advantage of iPSC technology is its ability to generate pluripotent cells from any person, regardless of their unique genetic makeup. This includes people with intermittent disease types and those afflicted with complicated multifactorial illnesses whose genetic makeup is unknown, like type 1 diabetes and liver cancers[64,65].

iPSC-derived exosomes for human heart diseases

In prior research, it was found that exosomes derived from embryonic stem cells aided in repairing damaged heart tissue after a heart attack. Similarly, exosomes from iPSCs showed promise in promoting lung and heart repair, protecting cells from oxidative stress, and enhancing various cellular functions. These iPSC-derived exosomes contain bioactive elements like mRNA, miRNA, and proteins that exert protective effects on recipient cells. Moreover, iPSC-based mesenchymal cells and their exosomes displayed potential for tissue repair and immunomodulation in several preclinical studies. The possible use of iPSC-based exosomes as a therapeutic tool for heart diseases is promising (Fig. 7). However, further research is required to comprehend the specific bioactive components in these exosomes, paving the way for safer and more effective treatments in the future[66].

Case study 1

The heart's limited ability to heal often leads to heart failure after injury. This study used iPSCs derived from organ-specific reprogrammed fibroblasts as a potential treatment. Researchers compared gene and protein expressions in different human iPSC sources and found 51 altered genes, notably miR22, a key regulator of heart function. Cardiac fibroblast-derived iPSCs (CF-iPSCs) showed lower miR22 levels than dermal fibroblast-derived iPSCs (DF-iPSCs). Experiments with exosomes from CF-iPSCs enhanced the differentiation of embryoid bodies (EBs) into

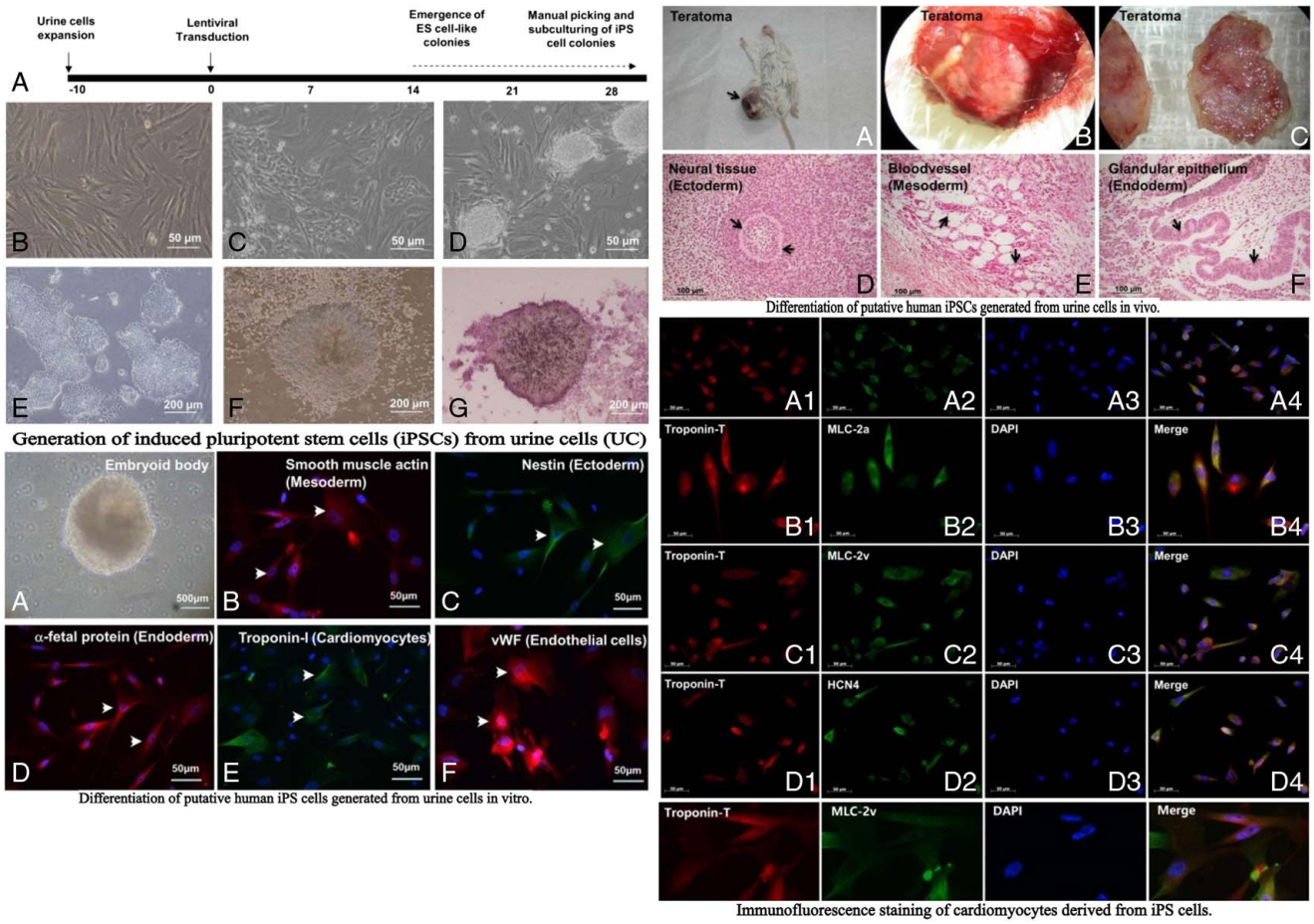

Figure 5. Generation of iPSCs, Differentiation of iPSCs *in vivo in vitro*, Immunofluorescence staining of iPSCs (adapted with permission under CCBY 4.0 from[62]). iPSC, induced pluripotent stem cell.

beating heart cells, suggesting their potential for heart recovery therapy. The reduced expression of miR22 means that exosomes from CF-iPSCs may not carry the memory of congestive heart cells, making them a hopeful biological source for potential treatments of heart injuries in the future[67].

Case study 2

The study conducted various experiments with cell cultures and differentiation processes. Embryonic stem cells and fibroblasts from mouse embryos were cultured and used for exosome isolation. Exosome properties like size were analyzed using dynamic light scattering and electron microscopy. Immunoblotting and immunohistochemistry techniques were utilized for protein analysis, while TaqMan Array MicroRNA and microRNA treatment were used to analyze and manipulate RNA. Oxygen consumption rates were measured in cells, and animal studies involving inducing acute myocardial infarction (MI) in mice, followed by treatment with exosomes, were conducted. Echocardiography was used to assess heart function post-MI. Result: The research discovered that exosomes originating from mouse embryonic stem cells (mES Ex) can potentially enhance heart function following a heart attack. These exosomes boosted the growth of new blood vessels, supported heart cell survival, and reduced scarring, leading to a resurgence of the heart's ability to regenerate.

Moreover, they elevated the survival, proliferation, and specialization of cardiac progenitor cells (CPCs) within the heart, leading to the generation of new heart muscle cells. The key factor behind these positive effects was the delivery of a specific microRNA, miR-294, from the embryonic stem cell exosomes to CPCs. This microRNA encouraged increased survival, progression through the cell cycle, and proliferation of these CPCs[68].

Case study 3

Involved deriving human iPSCs from human dermal fibroblasts via reprogramming and characterizing their properties for cardiac differentiation. A specific differentiation protocol yielded contractile tissue from iPSCs. Cardiovascular progenitor cells from the iPSCs were identified using various markers. The cells were also assessed for cardiac and endothelial potential. Additionally, iPSCs expressing enhanced Green Fluorescent Protein (eGFP) were generated and examined using a rat model simulating MI, followed by assessment through cardiac magnetic resonance imaging and histological analysis at different time points post-infarction to evaluate engraftment and functionality. Researchers have developed a groundbreaking monolayer technique to efficiently derive cardiac cells from human iPSCs. This method involved precise adjustments to growth factors and durations of exposure, resulting in the development of robust cardiac progenitors and functional heart cells. The protocol

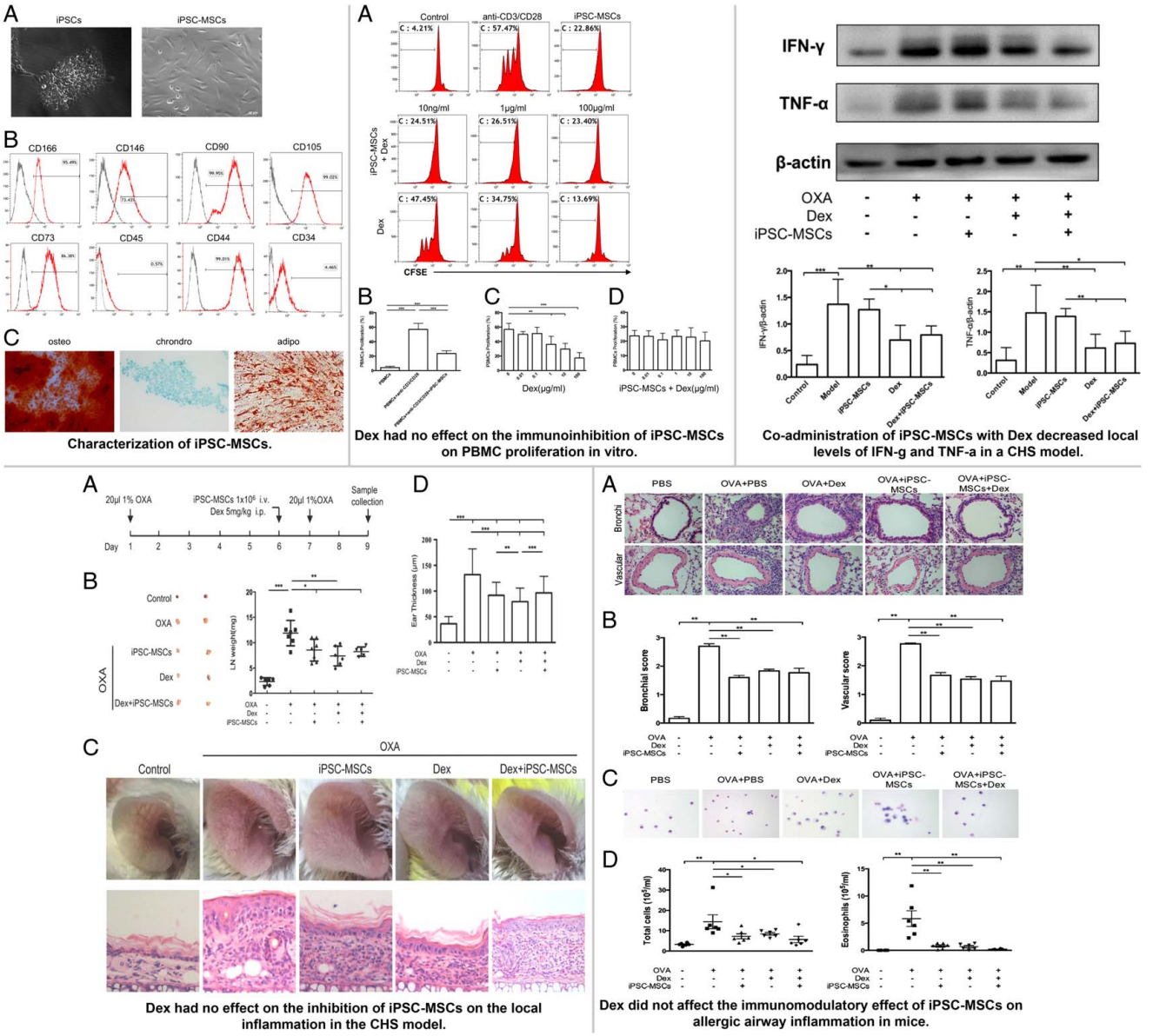

Figure 6. Characterization of iPSCs (adapted with permission under CCBY 4.0 from[63]). iPSC, induced pluripotent stem cell.

showcased robust biomarkers linked to cardiovascular lineage commitment and yielded cells displaying cardiomyocyte features. In a rat model mimicking heart injury, eGFP-labeled iPSC cells were introduced post-infarction. This revealed a trend toward preserved heart function compared to untreated infarcted hearts at 10 weeks. Histological analyses confirmed the presence of transplanted cells in the heart tissue, signifying their engraftment and potential role in preserving cardiac function after injury[69] (Fig. 8).

iPSC-based cell therapy for diabetic wound treatment

Treating non-healing diabetic wounds is tough, but using the cells belonging to the individual for therapy seems promising. With advancements in iPSC technology, there are new methods for tissue reconstruction. This implies that impaired tissue could be substituted with tailored, healthy tissue, effectively dealing with diabetic wounds. However, extensive animal testing is crucial before applying these iPSC-based treatments in humans. Directly converting one cell type into another without a middle pluripotent step could be a game-changer in research. This technique has shown potential in generating specific cells for personalized therapy across various cell types.

Additionally, a vital aspect of wound therapy involves regenerating skin layers and their components for complete functional recovery. Direct reprogramming-based cell therapy might assist in this process once it's refined. Improving in-vivo tissue reconstruction to mimic natural structure and function better is a future goal. Eventually, there's hope to convert individual cell types and transform different tissues—a significant leap for regenerative medicine[70] (Fig. 9).

Case study 1

The study created insulin-producing cells (IPCs) from iPSCs of Type 1 Diabetes, Type 2 Diabetes, and non-diabetic individuals.

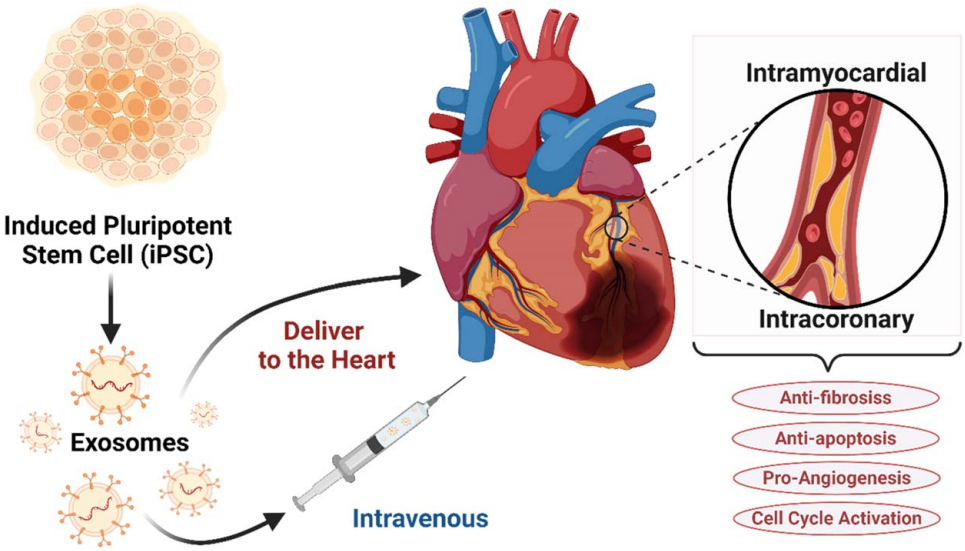

Figure 7. iPSC derived exosomes for human heart diseases. iPSC, induced pluripotent stem cell.

Using PDX1 gene-expression induction, IPCs from Type 1 Diabetes and Type 2 Diabetes iPSCs exhibited similar markers and insulin secretion levels as Non-Diabetes-iPSC-derived IPCs. This suggests the potential for using patient-specific iPSCs for autologous transplantation in Type 1 and Type 2 Diabetes. Notably, since Type 2 Diabetes affects a larger population and impacts insulin secretion over time, this study holds clinical significance. It's the first to compare IPCs generated from non-

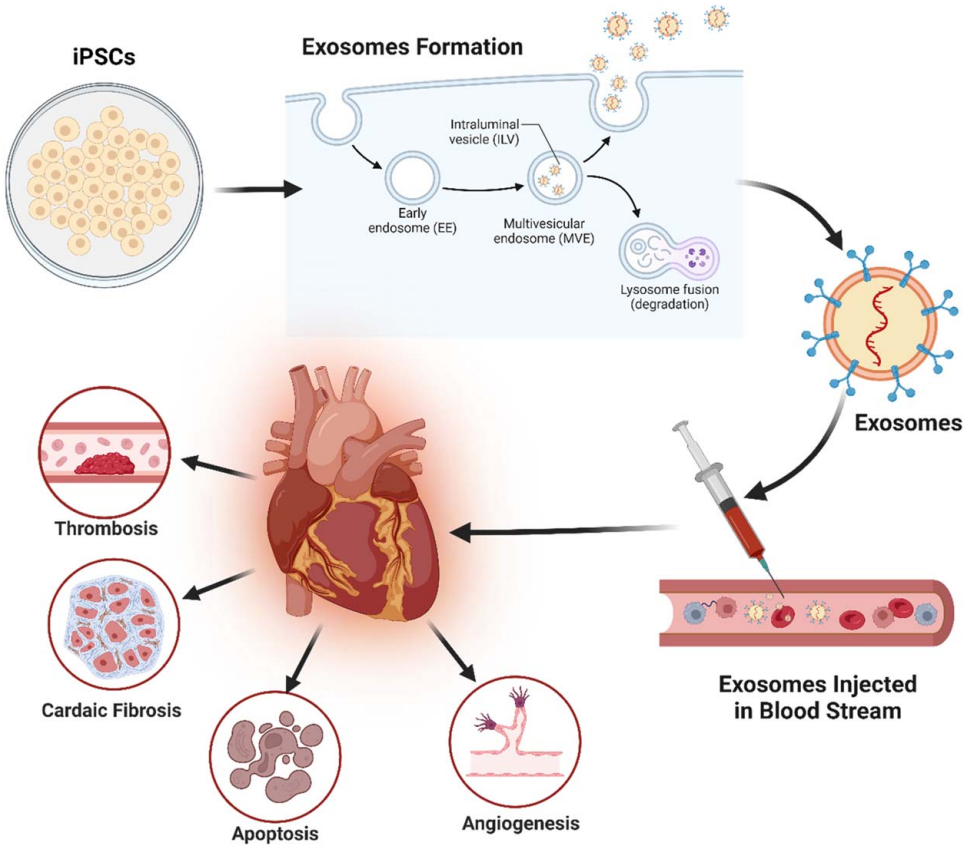

Figure 8. Therapeutic potential of iPSC-derived exosomes for heart disease. iPSC, induced pluripotent stem cell.

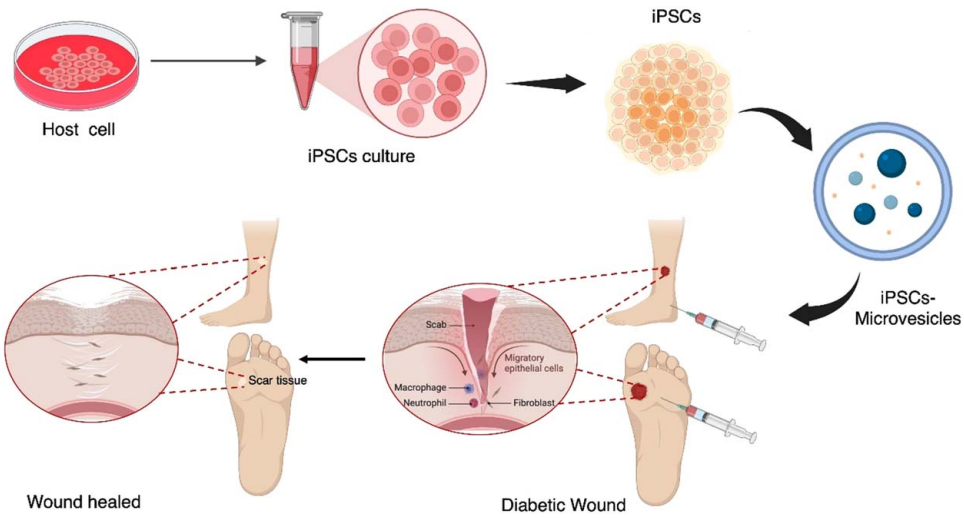

Figure 9. iPSC-based cell therapy for diabetic wound treatment. iPSC, induced pluripotent stem cell.

diabetic, Type 1 Diabetes, and Type 2 Diabetes iPSCs simultaneously, offering insights into diabetes treatment prospects[71].

Case study 2

In this investigation, researchers explored the healing capabilities of induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) in mouse wounds. They found that iPSC-EC treatment accelerated wound closure and increased blood vessel formation in the initial healing phase. There was a notable elevation in endothelial cell markers and enhanced wound perfusion during the first-week post-treatment. Moreover, iPSC-EC-treated wounds exhibited heightened collagen content by day 14, indicating advanced healing rather than excessive scarring. The treatment also led to sustained macrophage activity, possibly due to increased recruitment triggered by iPSC-ECs. However, the study noted a significant challenge in cell survival and integration after treatment, with most cells lost within 48 hours. Longitudinal tracking revealed a decline in iPSC-ECs over two weeks, indicating that their healing effects might primarily stem from secreted factors rather than cell integration or multiplication. Despite this, even minimal improvements in cell retention could potentially enhance tissue regeneration. To address this issue, further investigations involve studying iPSC-ECs on supportive biomaterial scaffolds to potentially prolong their presence and amplify their therapeutic impact[72].

iPSCs in autoimmune neurological disease—multiple sclerosis

While iPSCs have been thoroughly investigated for neurodegenerative and neurogenetic disorders, their exploration of inflammatory neurological conditions such as multiple sclerosis (MS) has been limited. Nevertheless, there is a clearer understanding of the pathway leading to nerve cell injury and death in MS compared to neurodegenerative conditions[73]. iPSC technology holds promise for potential therapeutic approaches, including regenerating specific nerve cell groups and exerting an immune-regulatory effect. Additionally, iPSCs provide more accurate disease models compared to animal studies. MS, a primary autoimmune condition affecting the central nervous system, is a chronic disorder marked by inflammatory episodes leading to neurological disability, especially in the remitting relapsing form. The causes of MS involve a combination of genetic predisposition and environmental factors. More than 50 susceptible regions have been identified through Genome-wide Association Studies (GWAS). Factors such as vitamin D3 deficiency and Epstein-Barr virus (EBV) infection contribute to the development of MS. Myelin-reactive CD4 + T cells and other immune cells play crucial roles in MS pathology. There is a high demand for the development of regenerative or immune-modulatory therapies for MS. Oligodendrocyte precursor cells (OPCs) derived from iPSCs offer a potential solution for remyelinating nerves after demyelination in MS, aiming to protect them from ongoing inflammation. Early remyelination might mitigate axonal loss, a significant cause of disability in MS. Animal studies have shown success in remyelination and disability improvement using iPSC-derived OPCs in an MS animal model. Furthermore, iPSC-derived neural precursor cells (NPCs) have demonstrated regenerative and immune-regulatory effects in MS models. When transplanted, these cells have shown neuroprotection by producing a specific neurotrophin, limiting central nervous system (CNS) inflammation, and subsequent tissue damage. The utilization of iPSCs obtained from an individual with MS has allowed the creation of nerve cells in a dish, revealing differences in their electrical behavior compared to healthy cells. This approach holds promise for uncovering new insights into MS pathology. In essence, iPSC technology presents potential solutions for MS treatment by offering cellular therapies, disease modeling, and a deeper understanding of MS pathogenesis[7] (Fig. 10).

Case study 1

Investigates neuroprotective mechanisms in benign multiple sclerosis (BMS) by comparing iPSC-derived astrocytes from BMS, progressive MS (PMS), and healthy controls. BMS astrocytes demonstrate a unique neuroprotective effect by activating JAK/STAT signaling through TNF-α/IL-17, producing factors such as LIF, BDNF, and TGF-β1. In contrast, astrocytes from PMS and healthy controls lack this neuroprotective ability. The JAK/STAT

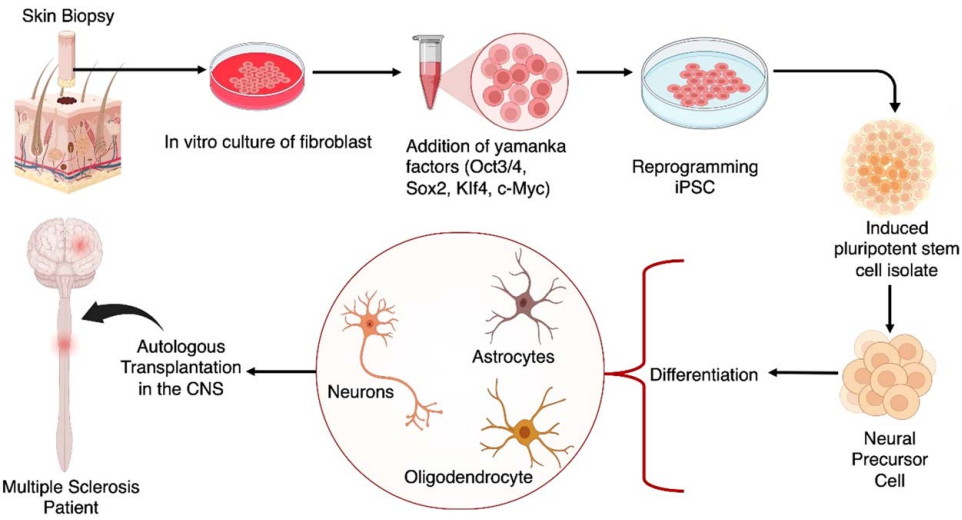

Figure 10. iPSCs in autoimmune neurological disease—multiple sclerosis. iPSC, induced pluripotent stem cell.

pathway, particularly JAK1 and JAK3, is enhanced in BMS astrocytes. Tofacitinib, a JAK inhibitor, diminishes the neuroprotective effect. This study highlights potential therapeutic targets for modulating CNS-endogenous pathways in MS patients with distinct disease course[74].

Case study 2

The study successfully derived functional neurons specific to MS patients from non-invasively collected renal proximal tubule epithelial cells. This signifies the initial dependable in-vitro human model for neurons affected by MS, making a valuable contribution to the expanding domain of humanized neurodegenerative disease models. Although contamination issues in the initial culture were encountered, the non-invasive collection method allows repeated collections without harm to patients. The procedure efficiently produced neural precursor cells and unique, long-lasting reservoirs of immature pre-neurons, leading to pure neuronal cultures. These neurons exhibited normal morphology and functionality, with physiological properties similar to healthy controls. Electrophysiological and morphological analyses showed no significant differences between MS patient-derived neurons and controls. The established MS model provides a platform for in-vitro studies on neuronal processes, drug screenings, and exploration of disease-associated genetic variations. The non-interventional approach provides a basis for exploring MS-specific aspects related to humans and neurons, including genomics and epigenomics[75] (Fig. 11).

iPSC application in Alzheimer's disease

The research dives into Alzheimer's disease (AD) exploration through iPSC modeling. The goal is to understand the influence of genetic mutations, unravel the mechanisms of the disease, and explore potential avenues for treatment[76]. The investigation spans various methodologies, encompassing neuronal analysis, diverse cell types, and three-dimensional models, aiming to unravel AD's intricate genetics and underlying processes. Familial AD neuronal modeling (fAD): Examining mutations in genes like Presenilin 1/2 and amyloid precursor protein (APP) within cortical neurons obtained from afflicted individuals, researchers found distinct AD features: elevated Aβ42/Aβ40 ratio, Aβ accumulation, and Tau level alterations. Detailed studies offered insights into how APP processing and endosome engagement contribute to disease progression. Sporadic AD neuronal modeling (sAD): Modeling sAD, especially with complex genetic backgrounds, proved challenging. While some sAD lines mirrored fAD traits, others resembled control conditions. The studies on APOE variants have highlighted diverse responses to treatments and fluctuations in Aβ and Tau levels. Precision editing in neuronal models, using CRISPR/Cas9 or TALEN systems, has allowed researchers to precisely edit genes, revealing unique effects associated with specific genotypes. By manipulating genes tied to AD mutations, researchers have gained insight into altered Aβ production, Tau pathology, and neuronal vulnerabilities. The exploration has expanded to encompass astrocytes, organoids, and 3D co-culture systems, offering a more comprehensive view of complex cellular interactions and a closer representation of AD pathology. These models have uncovered changes in cellular markers, Aβ build-up, and impaired cellular functions linked to AD. Human iPSC/Mouse Chimeric Model: By implanting human iPSC-derived neurons into transgenic AD mice, researchers unveiled human-specific contributions to neurodegeneration around Aβ plaques. This chimeric model provided a unique perspective on AD pathogenesis within a more humanized setting. Choudhary and colleagues address the possible advantages and drawbacks of integrating ChatGPT, an AI tool, into the teaching of veterinary anatomy. It outlines a number of benefits, including case-based learning, interactive learning, easily accessible reference materials, comparative anatomy exploration, visual representation of anatomical structures, and quizzes to reinforce important concepts. It also highlights some shortcomings, though, like the lack of some features in 3D models and the sporadic errors in the responses, which should be fixed in later releases. The letter highlights that ChatGPT should be used in conjunction with practical experiences and hands-on learning in veterinary education rather than as a replacement, despite its potential[77].

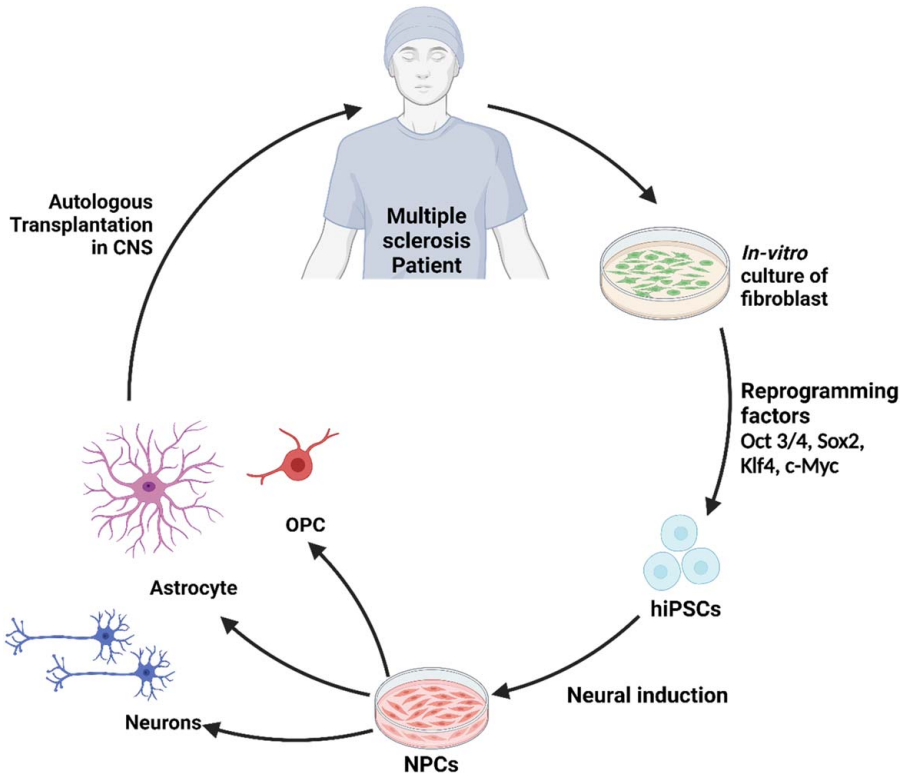

Figure 11. iPSC-based therapeutics for multiple sclerosis. CNS, central nervous system; iPSC, induced pluripotent stem cell; MS, multiple sclerosis; NPC, neural precursor cell; OPC, Oligodendrocyte precursor cell.

iPSC application in Tau-related neurodegenerative diseases

Studies involving mutations in the MAPT gene shed light on Tau pathology and its role in conditions like frontotemporal dementia. iPSC models unravel details about Tau accumulation, toxicity, and unusual cellular mechanisms related to Tau mutations or risk variants. In essence, iPSC-based models have emerged as invaluable tools in dissecting the intricacies of AD. These models aid in understanding disease mechanisms, testing potential therapeutics, and potentially guiding targeted treatments for Alzheimer's and related neurodegenerative conditions[78] (Fig. 12).

Case study 1

In this study, AD-patient iPSC-derived neurons were employed for a drug screening process to identify compounds that could reduce abnormal accumulation of phosphorylated tau protein (pTau), a characteristic feature of Alzheimer's disease. Of over 1600 compounds tested, 42 were discovered to effectively lower pTau levels, including known pTau modulators and newly identified compounds. The focus narrowed to cholesterol-targeting compounds, specifically statins, due to the known implication of cholesterol metabolism in Alzheimer's disease. Accumulation of cholesterol esters (CE), the storage forms of cholesterol, was observed in the brains of patients with AD and transgenic mouse models. The study demonstrated that reducing CE levels through various drugs acting via different mechanisms led to decreased pTau levels across multiple phosphorylation sites in neurons derived from familial AD (FAD), sporadic AD (SAD), and non-demented control (NDC) subjects.

Moreover, the research revealed that CE regulates pTau and impacts the secretion of amyloid-beta (Ab), another essential protein associated with Alzheimer's pathology. Remarkably, the impact of cholesterol ester (CE) on the production of Ab was found to be distinct from its effect on pTau levels. This implies that CE independently governs both pTau and Ab, signifying that these proteins are co-regulated by CE through separate pathways. These results underscore the notion that common upstream pathways, such as those involving CE in sporadic Alzheimer's disease, can elevate levels of both pTau and Ab through distinct molecular mechanisms. This suggests a more complex interplay between these hallmark proteins, challenging the notion of a singular linear pathway connecting Ab to Tau in the progression of the disease[79].

Case study 2

This research presents a human-derived AD model using iPSCs containing neurons, astrocytes, and microglia. Soluble Aβ42 species induced AD hallmarks through this triple-culture system, mirroring human disease progression. Early exposure primarily triggered synapse loss, whereas later stages involved plaque formation, tau protein hyperphosphorylation, and neuronal death. Pharmacological tests identified key pathways activated by Aβ42 species, correlating Aβ toxicity with tau pathology. The model's long-term culture captured age-related AD traits, distinct from brief cultures. This automated, scalable platform allows large-scale screening for neurodegenerative diseases, enabling potential

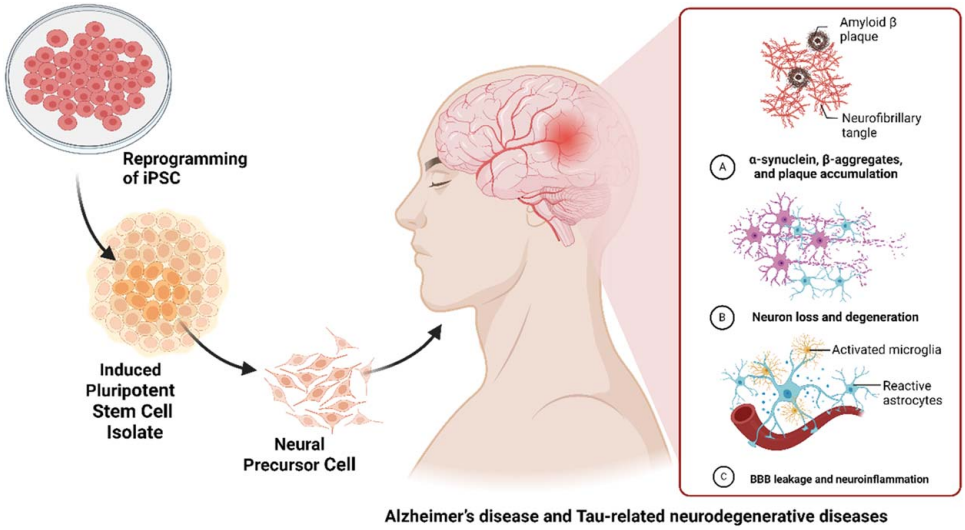

Figure 12. Human iPSC application in Alzheimer's disease and Tau-related neurodegenerative diseases. iPSC, induced pluripotent stem cell.

therapeutic target discovery. However, refining the model's tau aggregation and incorporating CRISPR techniques can enhance its translational potential, offering a more profound insight into the pathogenesis of AD and potential treatment strategies[80] (Fig. 13).

Role of iPSC in tissue regeneration and repair

Regenerative medicine involves the generation, substitution, or renewal of human cells, tissues, or organs. It employs biological techniques, tissue engineering methods, and stem cell therapies to restore regular function, specifically addressing age-related issues and physical or biological injuries[57] (Fig. 14). Maehr and colleagues derived iPSCs from patients type 1 diabetes through the reprogramming of adult fibroblasts using three transcription factors (OCT4, SOX2, KLF4). The generated iPSC demonstrated pluripotency and can mature into insulin-producing cells[81]. Wang and colleagues explored the potential link between missense mutations in the PDX1 coding region and diabetes mellitus. Two individuals with diabetes-prone tendencies had a reduced glucose tolerance and common missense mutations (P33T, C18R) in the PDX1 coding region when the researchers studied groups with high risk of diabetes. iPSCs were derived from these patients, and isogenic cell lines with various mutations were created. The findings revealed that these mutations negatively impacted beta-cell differentiation and function, along with the efficiency of pancreatic progenitor differentiation. Mechanistically, the mutations caused a reduction in key genes associated with insulin synthesis and secretion, offering insights into the role of PDX1 mutations in diabetes susceptibility[82].

Wernig and his colleagues demonstrated the therapeutic value of replacing neurons with reprogrammed fibroblasts in animal models. iPSC cells were efficiently transformed into neural precursor cells, which, when transplanted into the fetal mouse brain, differentiate into various neuronal and glial cell types. The transplanted neurons exhibited enhanced activity and integrated into the host brain, suggesting potential for neuronal replacement. Furthermore, iPSCs were stimulated to develop dopamine neurons, which could benefit treating Parkinson's disease in a rat model[83]. Uchida and colleagues created iPSCs lines from bone marrow stromal cells (MSCs) and erythroid progenitors (EPs) obtained from the peripheral blood of individuals with sickle cell disease. MSC-derived iPSC sacs demonstrated enhanced generation of immature hematopoietic progenitors and definitive hematopoietic stem/progenitor cells, leading to enhanced generation of erythroid cells with increased expression of β-globin[84]. However, a microenvironment or niche is essential to promote regeneration since it impacts the cell's behavior during development and repair. Regenerative medicine using iPSCs offers clinical advantages. Patient-derived iPSCs, when transplanted back to the same individual, are immunologically privileged, potentially eliminating the need for lifelong immunosuppressive drugs. Moreover, iPSCs possess the capacity for self-renewal and proliferation akin to ESCs, rendering them a plentiful and inexhaustible cell source for use in cell replacement therapy within the field of regenerative medicine[85].

Mesenchymal stem cell (MSC) therapy shows promise for treating spinal cord injury (SCI) by aiming to restore motor and sensory function. However, the efficacy of MSCs in both preclinical studies and clinical trials varies, likely due to factors such as SCI neuropathology and MSC source and dosage. Transplanted MSCs are vital in SCI treatment as they provide neurons and glial cells and create an optimal environment for neuroregeneration and angiogenesis at the injury site. MSCs achieve immunosuppression by interacting with immune cells or releasing signaling molecules, thus reducing inflammation. Additionally, they release neurotrophic factors supporting axonal regeneration, regulate pathways inhibiting glial scarring, and enhance angiogenesis. Despite improvements in sensory and motor scores demonstrated in studies, the overall effectiveness of MSC therapy for SCI is not yet sufficient for widespread clinical use. Challenges such as the hostile environment associated with SCI threatening MSC survival, uncertainty regarding optimal cell dose and frequency, MSC mechanism of action, and inhibitory cellular processes hindering neural circuit recovery require further exploration[86]. Regenerating nerve cells in the injured spinal cord faces several challenges, including extensive cell loss, limited

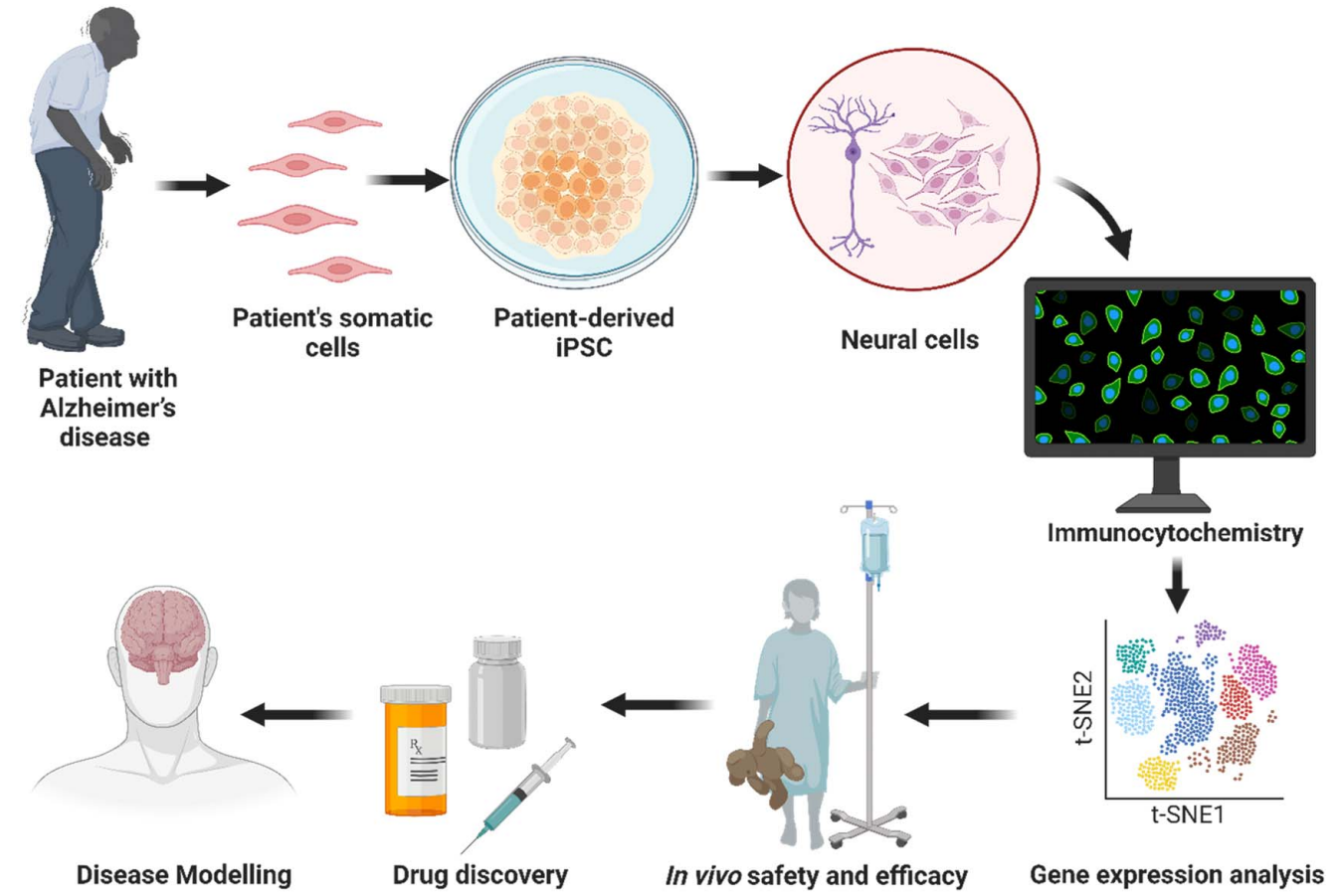

Figure 13. Contribution of iPSCs in Alzheimer's disease. AD, Alzheimer's disease; iPSC, induced pluripotent stem cell.

neural cell regeneration capacity, axonal disruption, and the presence of growth-inhibiting molecules, particularly astroglial scarring or glial scars at the injury site in chronic cases[87].

iPSC in drug screening

Numerous medications do not reach the market due to unforeseen adverse effects in humans, even after successfully passing animal tests. The early anticipation of toxic effects in humans during drug development has the potential to lower costs, but the difficulty lies in the limited and unstable availability of human samples[88]. Human iPSCs provide a resolution to this challenge. iPS cell technology offers a significant advancement in pharmacological and toxicological screening. It enables the development of human cell types specific to certain diseases, enhancing the accuracy of testing therapeutic responses and toxicity. Generating different iPS cell lines for a particular illness facilitates the examination of genetic and potential epigenetic differences within a varied population.

Additionally, it facilitates investigating the personalized therapeutic effects of drugs at an individual level, supporting the concept of personalized medicine based on a patient's genetic or molecular profile[89]. The technology of iPSCs can substantially decrease the need for sacrificing animals in drug testing, enable the early identification of human toxicity in preclinical trials, and reduce the risks and expenses associated with clinical trials[64]. While iPS technology benefits drug screening, it's crucial to recognize that cells originating from iPS cells might be developmentally immature, resembling fetal biology. To correspond with the biological characteristics of adult humans in drug development, it is crucial to prompt maturation in cells derived from iPSC before employing them. Creating lineage-specific reporter lines and other genetic manipulation techniques makes it possible to differentiate and mature iPS cells into specific cell types, enhancing their drug-testing ability[90].

Blastocyst and iPSC applications

Addressing the organ donor shortage, bioengineered organ transplantation stands out as a viable solution. Progress toward human transplantation involves generating swine-based scaffolds for compatibility with human organ sizes or human iPSC-derived organoids. However, promising, developing entire organs suitable for clinical application remains elusive. Overcoming the limitations of decellularized organs and organoids requires conceptual and technological breakthroughs. The BC method presents prospective advantages, particularly in terms of scalability, livestock utilization, and its surgical applicability to organ transplantation for various end-stage refractory diseases, differentiating it from organoid and decell-recell approaches[91].

Deng and colleagues, in their editorial, reported that the blastocyst complementation approach shows significant potential

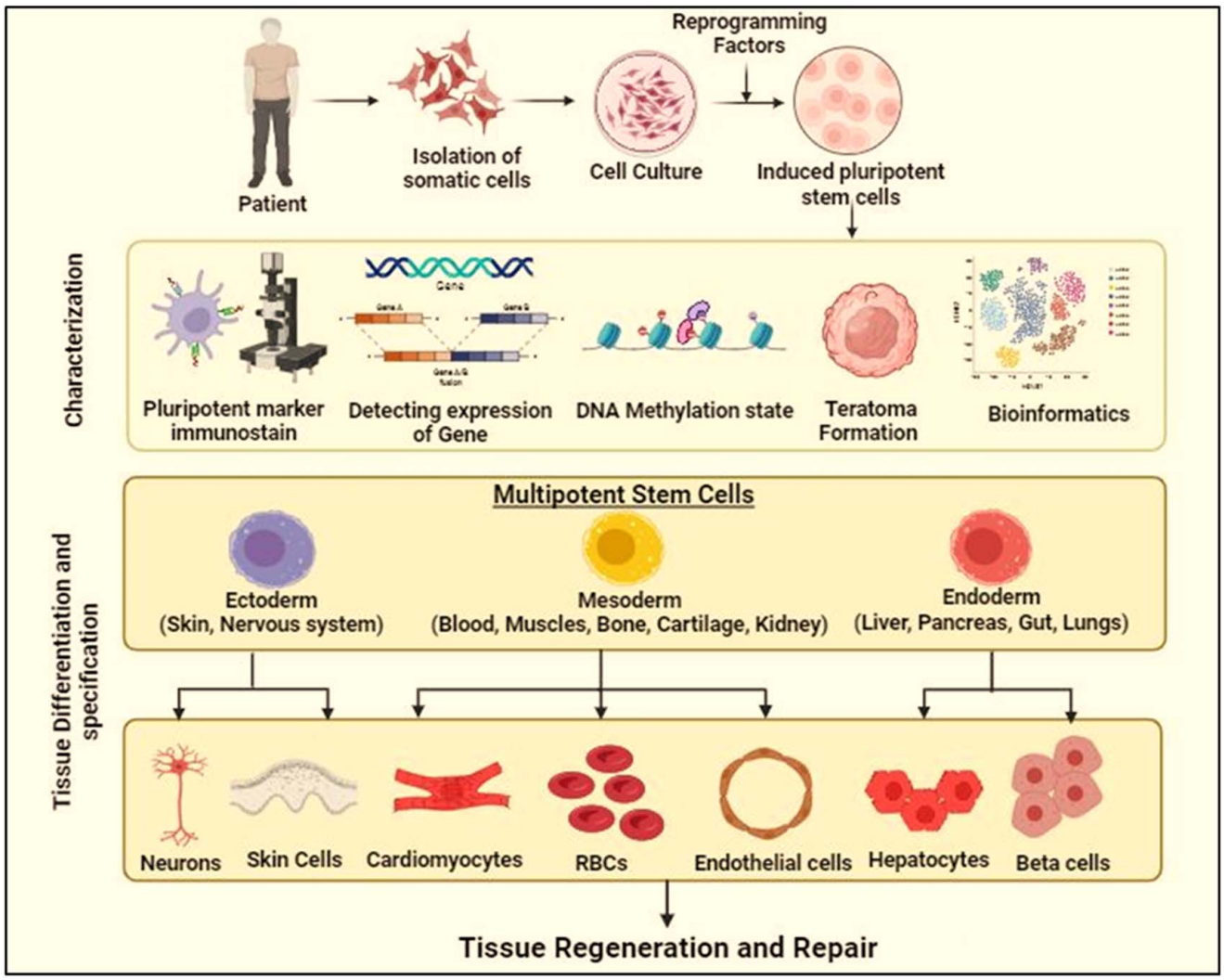

Figure 14. Induced pluripotent stem cell in tissue regeneration and repair. RBC, red blood cell.

for organ generation, emerging as a promising and easily accessible resource for cellular therapies. This innovative method holds revolutionary promise as a viable treatment for various terminal diseases. Leveraging the regenerative capacity inherent in this approach, it offers a groundbreaking avenue for developing radical treatment options, potentially transforming the landscape of medical interventions for conditions considered otherwise incurable[92]. Whole organ generation through blastocyst complementation is a promising avenue for cellular therapies and radical treatment for terminal diseases. However, challenges like organ size scalability, immune system incompatibilities, long-term maintenance, and evolutionary distance between donor and host cells persist. A multifaceted approach is needed to address these challenges, particularly in understanding the mechanisms of interspecies chimerism formation. Recent research, summarized by Choe *et al.*[93] and Sarmah and colleagues, provides insights into the history of interspecies chimerism and outlines the challenges and prospects of blastocyst complementation for human organ generation. Swine models are up-and-coming for xenotransplantation, with the first porcine cardiac xenotransplantation performed in early 2022. Gene editing techniques have been used to engineer porcine vasculature, paving the way for human-porcine chimeric organ production. Initial studies involving human-porcine chimeric embryos have shown promise, but further research is needed to understand developmental progression, recipient animal immunological responses, and strategies to enhance efficiency. Despite these challenges, there is significant enthusiasm for the potential of blastocyst complementation to revolutionize organ transplantation and treat end-stage diseases[92].

Benchetrit and colleagues successfully reprogrammed fibroblasts into iPSCs, induced trophoblast stem cells (iTSCs), and induced extraembryonic endoderm stem cells (iXENs) using Gata3, Eomes, Tfap2c, Myc, and Esrrb. Their sophisticated knockin reporter system revealed the simultaneous induction of these cell types. Transcriptomic and epigenetic analyses highlighted the crucial role of Esrrb and Eomes interplay, where high Esrrb levels induced pluripotency, while elevated Eomes levels directed trophectodermal fate. This groundbreaking study provides valuable insights into cellular reprogramming mechanisms and offers potential applications in regenerative medicine[94].

Safety considerations

Installing a "safety switch" like the iCASP9 system into human pluripotent stem cells enhances safety for potential therapies. By precisely integrating it into a genomic safe harbor, the AAVS1 locus, and using the CAG promoter for expression, we achieve strong and stable iCASP9 expression. This allows for the efficient elimination of stem cells and their derivatives upon activation with AP1903, minimizing safety concerns associated with uncontrolled cell replication or activity[95].

The potential of human iPSCs in biomedical sciences, particularly for regenerative medicine and disease modeling, is vast. However, safety concerns, including genetic and epigenetic abnormalities, tumorigenicity, and immunogenicity of transplanted cells, underscore the need for rigorous safety measures in iPSC-based therapies. Despite these concerns, accumulating preclinical data are demonstrating the safety and efficacy of iPSCs. One of Hideyuki Okano's reviews focuses on recent advancements and future challenges in ensuring the safety of iPSC-based therapies, using repair strategies in the damaged central nervous system (CNS) and cardiovascular system as models. Understanding and addressing these safety concerns are critical for realizing the full potential of iPSC-based cell therapy in clinical settings[4].

The risk of transformation to undesired cell lines, whether benign or malignant, is a significant concern in stem cell therapy within precision medicine. While stem cells hold immense potential for regenerative treatments, their ability to differentiate into various cell types can pose risks if not tightly controlled. In iPSC-derived allogeneic therapies, donor selection and gene editing are performed once over the lifetime of the product, as opposed to being part of the manufacturing of each product batch. The introduction of a well-characterized, fully modified, clonally derived master cell bank reduces risks that have been inherent to primary cell-derived autologous and allogeneic therapies[96].

According to recent research, there may be a risk of tumor development from genetic abnormalities in stem cells, especially iPSCs, especially when autologous hiPSC-derived cells are involved. These anomalies, which differ from the genomic profiles of the original cells and can occur during reprogramming as a result of stress and oncogenic agents, include copy number variations (CNVs) and single-nucleotide polymorphisms (SNPs)[97]. HPSCs undergo genetic adaptations during culture, potentially affecting safety. Monitoring genetic stability is crucial using methods like genotyping or whole-genome sequencing. Banking PSCs at low passages reduces the risk of genetic changes. Epigenetic differences exist between PSC types, impacting their utility. Mitochondrial DNA integrity in PSCs requires further investigation due to potential mutagenesis during reprogramming[98]. Immune reactions may be elicited by administering stem cells, which could impact the recipient's immune system in addition to the cells themselves. Although MSCs and ESC-derived cells are thought to be less immunogenic, their potential can be affected by the site of administration and differentiation state. MSCs have demonstrated immunomodulatory effects both *in vivo* and *in vitro*, raising concerns about possible adverse effects and immunological suppression as well as possible therapeutic benefits. Overall, a number of factors, such as cell type, manipulation techniques, and immune responses, must be carefully taken into account in order to comprehend and reduce the risks associated with stem cell therapy. To increase the safety and effectiveness of stem cell-based therapies, more research is required[99].

Clinical trials

iPSC therapies have significant potential for treating a wide range of therapeutic disorders. Initially, hESCs were used for several studies, with clinical trials showing some challenges, such as immunological rejection in patients post-injection. In 2006, the iPSC term came into light as a therapeutic approach for diseased conditions. Kim and colleagues give an insight into the ongoing clinical trials for iPSC. Initially, comparisons were made between interventional and observational studies and later with therapeutic and non-therapeutic trials. The observations were made on global distributions, target size, type, and disorder purpose. The USA, with 187 participants, was known to have conducted major trials[100]. The ongoing clinical trials on the iPSC-derived cells are tabulated in Table 4 (https://cynata.com/osteoarthritis, https://www.cira.kyoto-u.ac.jp/e/pressrelease/news/231226-090000.html, https://clinicaltrials.gov/).

Challenges and concerns

iPSC therapies show considerable potential for treating diverse diseases, but they present notable challenges. The complex production process includes obtaining somatic cells, cellular reprogramming, expanding iPSCs, establishing a cell bank, and demanding reproducibility, efficiency, and compliance with Good Manufacturing Practice standards. However, the extensive in-vitro manipulation introduces higher risks[14]. The choice between autologous and allogeneic therapies poses dilemmas[101]. Autologous iPSC therapies, which rely on the patient's cells, can be costly and time-consuming, with challenges exacerbated by rigorous testing processes[102]. Legal and ethical considerations, such as concerns about somatic cell sources and potential embryo development, add complexity[14]. Furthermore, the limited clinical experience of iPSC-derived cells highlights significant challenges that must be addressed for safe therapeutic use. These obstacles emphasize the pressing need for further research and development in iPSC-based therapies[103].

Specialist organizations such as the International Society for Stem Cell Research (ISSCR) have independently developed or updated protocols for using stem cells in cell therapy, with input from experts worldwide. These guidelines highlight important ethical, legal, and social aspects of cell therapy. They cover manufacturing conditions, characterization of clinical-grade cells, confidentiality of genetic material and personal information, informed consent, genetic manipulation of cells, and issues related to intellectual property and patents, among other important considerations[14].

Conclusion and future perspective

The use of iPSC-derived cell therapy in regenerative medicine has brought both remarkable achievements and ongoing challenges. This therapy has revolutionized the medical field by using iPSCs to create different cell types for regenerative purposes. It holds promise for tissue regeneration, disease modeling, and personalized drug screening. While there have been success stories,

Table 4

Ongoing Clinical trial on iPSC-derived cells.

Trial name	Condition	Therapy type	Phase	Location	Status	Trial number/UMIN ID
Therapeutics: CYP-004	Osteoarthritis	iPSC-derived MSCs	Phase 3	Multi-national	Recruiting	ACTRN12620000870954
Kyoto University	Parkinson's disease	iPSC-derived dopaminergic cells	Phase 1/2	Japan	Ongoing	UMIN000033564
NIH Clinical Center	Dry age-related macular degeneration (AMD)	iPSC-derived retinal cells	Not specified	Bethesda, Maryland, USA	Recruiting	NCT02564978
CiRA Foundation	Heart failure	iPSC-derived cardiomyocytes	Phase 1/2	Japan	Ongoing	NCT0087588
Fate Therapeutics: FT819	Hematologic malignancies	iPSC-derived CAR-T cells	Phase 1	USA	Recruiting	NCT04629729
Asahi Kasei	Ischemic stroke	iPSC-derived neural cells	Phase 3	Japan	Ongoing	UMIN000000758

iPSC, induced pluripotent stem cell; MSC, mesenchymal stem cell.

challenges such as potential adverse effects and the need for careful ethical and regulatory navigation still exist. Looking to the future, advancements in technology and our understanding of cellular mechanisms are expected to overcome these challenges. Collaborative efforts across disciplines will be necessary to address concerns related to tumorigenesis and scalability. Additionally, regulatory frameworks will need to evolve to ensure the ethical and responsible translation of iPSC-based therapies from research to clinical use. Future research should focus on refining iPSC-derived cell therapies, identifying disease-specific nuances, and expanding the range of treatable conditions. Innovative modifications, such as using CRISPR and other genome-editing technologies, may improve the accuracy and safety of iPSC-based interventions. The collaboration of scientists, clinicians, ethicists, and regulatory bodies will play a crucial role in shaping the future of iPSC-derived cell therapy.

In conclusion, iPSC-derived cell therapy represents a pursuit of groundbreaking medical solutions. As we navigate through challenges, the profound impact of regenerative medicine and personalized therapy becomes increasingly apparent. The legacy of iPSCs in medicine promises a future where these cells contribute to a new era of healing and hope.

Ethical approval

Not applicable.

Consent

Not applicable.

Source of funding

The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work through Large Research Project under grant number RGP2/406/45.

Author contribution

P.M., A.P., B.D.: study concept and design; R.D., A.B., S.M.: data collection; T.A.A., H.A.S., A.K.: data interpretation; P.M., A.P., B.D., R.D., S.M., S.B.G.: writing the paper and editing.

Conflicts of interest disclosure

The authors declare no potential conflict of interest exists.

Research registration unique identifying number (UIN)

Not applicable.

Guarantor

Ajoy Kumer.

Data availability statement

No new data are generated.

Provenance and peer review

Invited Special Paper for the special issue "Evolving trends in stem cell therapy".

Acknowledgements

The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work through Large Research Project under grant number RGP2/406/45.

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