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Roles of iPSCin Advancing Treatments for Neurological Disorders

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ABSTRACT

Historically, disease research in neuroscience faced limitations due to species barriers and constraints with human samples. The advent of iPSC-induced neurons represents a significant breakthrough for academic and pharmaceutical research. Interdisciplinary collaborations have played a pivotal role in advancing iPSC technology. These collaborations have yielded innovative technologies, enhanced our understanding of cellular interactions and expanded iPSC applications. Despite progress, challenges persist, including concerns about tumorigenicity, immune rejection, inherent heterogeneity, and difficulty in recapitulating sporadic diseases. Scalability issues and the absence of age-associated cellular features in iPSC models are notable drawbacks. This concise review examines recent advancements in iPSC technology, focusing on its application in addressing neurological diseases. It delves into iPSC integration in the pharmaceutical industry, emphasizing roles in disease modelling, drug discovery, and screening. Looking ahead, the exploration of innovative strategies, interdisciplinary collaborations, and emerging technologies will continue shaping iPSC applications in neuroscience and the broader biomedical landscape. The systematic analysis and presentation of newly developed techniques in this review offer a roadmap for overcoming obstacles, emphasizing the transformative impact of iPSC technology on academic research and pharmaceutical development, and providing new avenues for understanding, treating, and potentially curing neurological diseases.

Keywords: Induced pluripotent stem cells, Cell replacement therapy, Drug discovery, Disease modelling, Autologous cell therapy, Allogenic Cell therapy, Clinical trials with stem cells, Neurological disorders, Neurodegenerative diseases

INTRODUCTION

The intricate domain of neurological disorders spans a diverse array of over 600 pathologies that affect the human central or peripheral nervous system, causing behavioural, emotional, and cognitive impairments. The global health landscape is confronted with significant challenges owing to the morbidity and mortality associated with neurological disorders. More importantly, many common neurological disorders are considered sporadic and triggered by a combination of genetic and environmental risk factors, such as aging. Therefore, the escalating global demographic shift towards an aging population further amplifies the societal burden imposed by neurological disorders [1]. Unfortunately, most neurological disorders remain incurable owing to limitations in diagnostic methods and our understanding of disease mechanisms. Conventional neuroscience investigations rely on animal models or patient-derived cell samples; however, these methodologies are encumbered by inherent limitations such as interspecies disparities, constrained sample volumes, and ethical considerations. The advent of Induced Pluripotent Stem Cell (iPSC) technology has emerged as a transformative modality, heralding the promise of robust disease modelling, pharmacological screening, and neural tissue regeneration, thereby propelling the prospect of efficacious interventions for a myriad of neurological disorders.

The breakthrough establishment of iPSCwas established by the Yamanaka group in 2006, which successfully reprogrammed murine embryonic or fibroblast cells into pluripotent stem cells through the induction of four critical pluripotency factors: Oct3/4, Sox2, c-Myc, and Klf4y e [2]. This ground-breaking discovery has marked a new era in stem cell research. iPSCs, akin to embryonic stem cells (ESCs), can differentiate into any cell type in adults. Notably, iPSChave more advantages over ESCs, including easier genetic modification, diminished ethical issues, diverse cell sources, superior reproducibility, and scalability. The versatility of iPSCs is underscored by their ability to differentiate into various cell types within the nervous system, including diverse types of neurons, oligodendrocytes, astrocytes, and microglia (Table 1).

Table 1 Summary of pluripotent stem cell differentiation protocols into nervous system cells across relevant publications

Cell Type	Cell Source	Reprogramming Factors	Reference
Neuron (e.g. motor neurons, peripheral sensory neuron, DA neuron, etc.)	Pluripotent stem cells (iPSCs or ESCs)	Morphogens (e.g. WNT, SHH, BMP, etc.)	[3- 6]
Oligodendrocyte Progenitor Cells (OPCs)		SHH, Growth Factors (e.g. PDGF, etc.)	[7-13]
Astrocytes		RA, FGFs, SHH, etc.	[15-19]
Microglial		CSF1, IL34, etc.	[20 -25]

iPSCIn Tackling Neurological Disorders

Decades in iPSCtechnology development have elevated its prominence as a pivotal tool in cellular neuroscience. Significantly, it has paved the way for addressing neurological diseases by propelling advancements in disease modelling, facilitating drug discovery and screening processes, and advancing progress in cell therapy [26-30] (Figure 1).

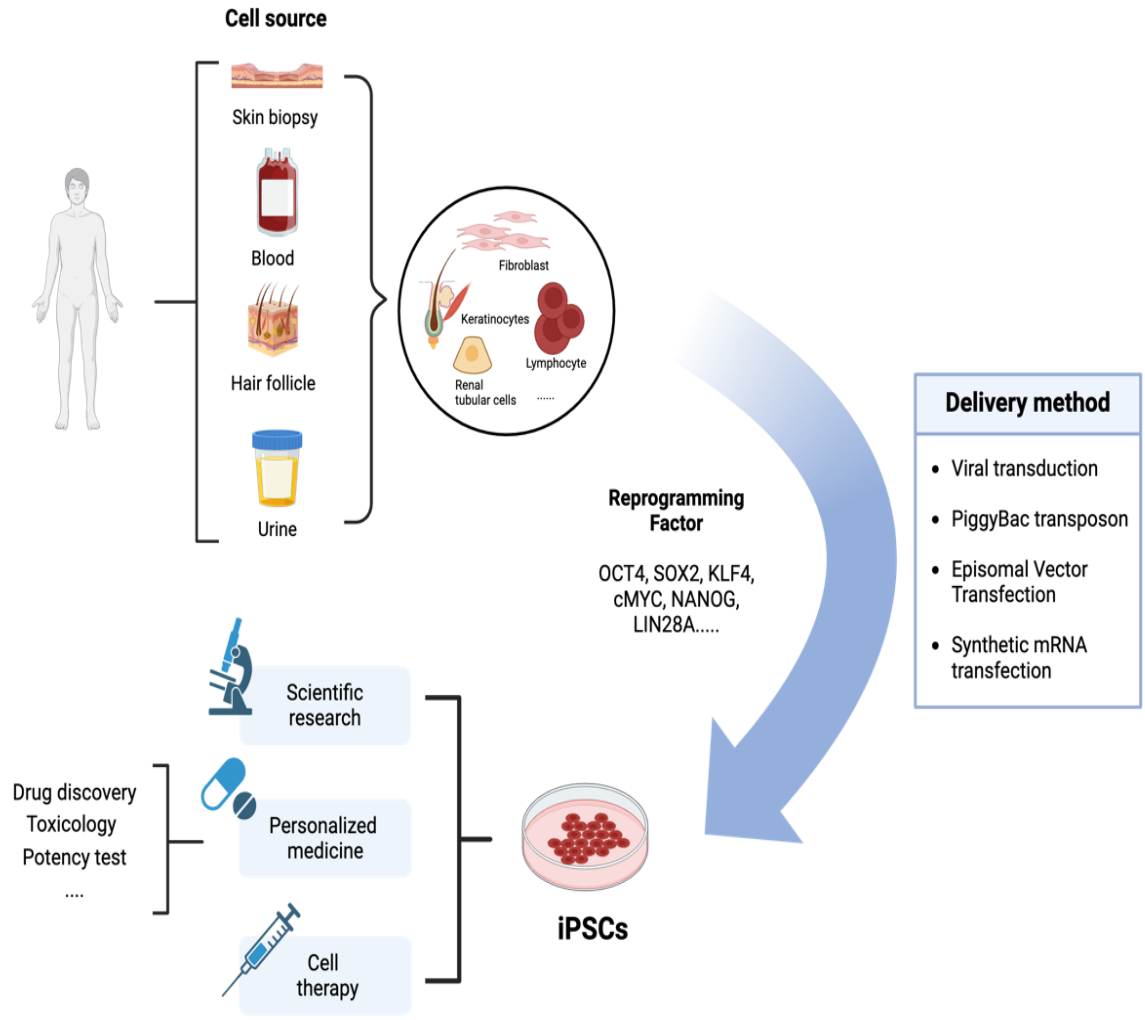


Figure 1 Overview of iPSC cell line generation and applications in neurological disease treatment

iPSCin Disease Modelling

iPSC technology has been extensively employed to generate pathological models of diverse neurological disorders, including Parkinson's Disease (PD), Alzheimer's Disease (AD), Amyotrophic Lateral Sclerosis (ALS), Multiple Sclerosis (MS), Schizophrenia, Huntington's disease. Most neurological diseases exhibit a sporadic nature, characterized by intricate etiologies involving complex genetic backgrounds and diverse epigenetic risk factors, encompassing environmental influences and the aging process. This inherent complexity poses considerable challenges to drug discovery and therapeutic development. iPSC technology initially focused on modelling monogenic familial diseases [31-39]. However, advancements in gene editing techniques and their integration with bioengineering technologies have led to the establishment of innovative methodologies such as organ-on-chip systems and organoids. This progress has enabled a seamless transition of iPSC methodology to the study of sporadic neurological disorders. Currently, iPSC technology is effectively applied to model complex disorders that lack clearly identified causative genes. The subsequent chapter will delve into the detailed exploration of the applications of iPSC technology in the treatment of representative neurological disorders (Figure 2) [40-51].

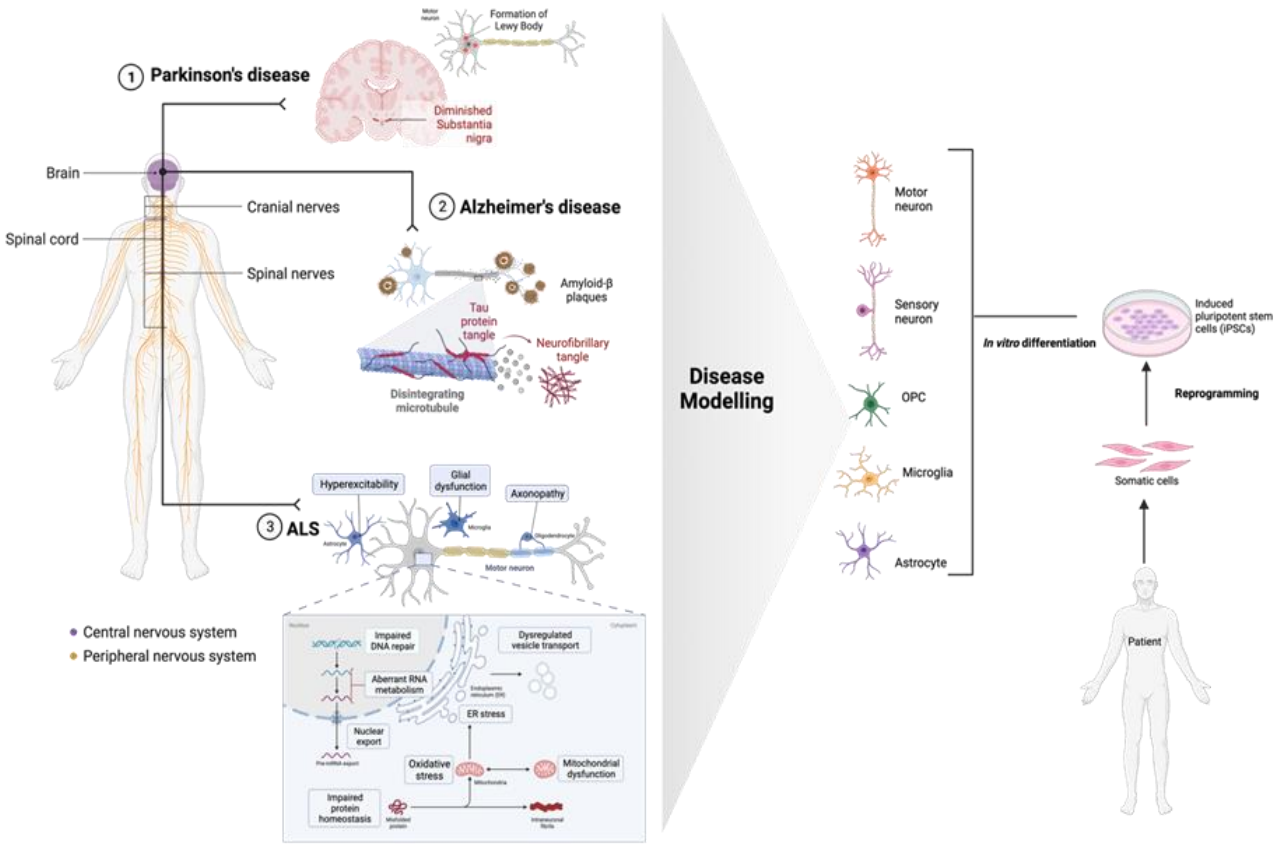


Figure 2 Summary of the application of patient iPSC-derived neurons in modelling neurodegenerative diseases

Parkinson's Disease (PD)

Parkinson's Disease (PD) is the second most prevalent neurodegenerative disease worldwide. The underlying pathology manifests through the depletion of dopaminergic neurons in the substantia nigra region of the brain, with the pivotal involvement of misfolded and aggregated proteins (α-synuclein) deemed critical in the neurodegenerative cascade [52-65]. More than 90% of PD cases are sporadic SPD, with no clear gene linkage, whereas the remaining cases, classified as familial PD (FPD), exhibit inheritable traits. Noteworthy FPD-associated genes include PARK1/PARK4 (α-synuclein [SNCA]), PARK2 (PARKIN), PARK6 (PTEN-induced kinase 1 [PINK-1]), PRAK7 (DJ-1), and PARK8 (Leucine-Rich Repeat Kinase 2 [LRRK2]), which also contribute to the pathogenic mechanisms of SPD [64-66]. The exact etiology and pathology of PD remain incompletely understood primarily because of the scarcity of research samples. Previous research in this area has relied on brain samples from patients, which is very limited. A noteworthy advancement has emerged in contemporary disease research through the widespread utilization of iPSC-derived neurons harboring PD-related mutation backgrounds. iPSChave been used for modelling PD pathology for more than 10 years, originating from the pioneering work by Park et al. in 2008 [67]. The ongoing maturation of iPSCtechnology has led to the development of diverse iPSC-derived PD models [68].

For example, SNCA A53T mutation and triplication-carrying iPSC-derived DA neuron cell lines were generated with increased α-synuclein concentrations detected [69-71]. Many other cell types, such as neuronal precursor cells, have also been generated from SNCA mutation-carrying iPSC. These models provide insights into many pivotal molecular mechanisms of FPD pathology, including disrupted synaptic connectivity, Endoplasmic Reticulum (ER) dysfunction, and altered oxidative stress vulnerability [72-75]. Despite the absence of clear genetic mutation sites in SPD, successful establishment of an SPD iPSCmodel has been achieved. This model was initially developed using skin biopsies of patients with SPD via the application of a modified lentivirus [76]. A comprehensive study of disease-specific phenotypes was conducted through a comparative analysis of cellular variation between iPSCderived from SPD patients and their healthy counterparts [77-79]. Apart from DA neurons, mounting evidence indicates that dysfunction of various Central Nervous System (CNS) cell types, including astrocytes, contributes significantly to PD pathogenesis, particularly in FPD [80]. Consequently, iPSC-derived astrocytes from patients with PD have become integral to research in this field. Recent advancements in iPSCreprogramming have led to the development of innovative disease modelling tools. For instance, the omics-hiPSCmodel is a potent technique for identifying the prognostic biomarkers of diseases [81]. An advanced human iPSC-based preclinical model for Parkinson's disease with optokinetic alpha-synuclein aggregation has also been developed [82]. Additionally, neural chimeras derived from DA neurons differentiated from either human or nonhuman primate iPSCand engrafted into rodent brains have proven valuable in modelling PD. In addition to modelling, autologous transplantation of primate iPSC-derived neurons has demonstrated successful survival in the striatum of primate recipients without the need for immunosuppression [83].

Alzheimer's Disease (AD)

iPSCtechnology has emerged as a highly promising tool for modelling Alzheimer's Disease (AD), the most common neurodegenerative disease. The pathological hallmarks of AD include extracellular decomposition of amyloid β-peptide (Aβ) plaques and intracellular formation of Neurofibrillary Tangles (NFTs) in the cerebral cortex [84]. These aberrant aggregations profoundly disrupt neuronal interactions, giving rise to progressive symptoms including dementia, speech or motor impairment, and cognitive issues. Similar to other neurodegenerative diseases, AD can be categorized into two groups: early onset Familial AD (FAD) and late-onset Sporadic AD (SAD). Although over 90% of AD cases are sporadic, no causative mutations have been definitively identified for SAD. Nonetheless, numerous associated genetic loci, such as the APOE gene, which encodes a lipid carrier that clears Aβ amyloid in the brain, have been identified [85-89]. Rodent models have been well-established for both FAD and SAD over the past few decades. However, despite more than 30 years of investigation in this area, only a limited number of effective clinical trials have been conducted for unknown reasons. iPSCtechnology offers a promising avenue for AD research by providing a human cell model that can differentiate into both neuronal and non-neuronal cells involved in AD pathology, including oligodendrocytes, pericytes, and vascular endothelial cells. Several studies have demonstrated the utility of iPSC-derived cells carrying FAD mutations to produce an increased number of Aβ plaques [90,91]. Furthermore, the development of a 3D co-culture model and organoids facilitated a more detailed and prolonged examination of the abnormal protein aggregation process. This approach is invaluable for in vitro studies of disease progression mechanisms, offering a comprehensive understanding of AD pathology over extended timescales [92,93].

Amyotrophic Lateral Sclerosis (ALS)

ALS is a neurodegenerative disease characterized by progressive muscular weakness and paralysis that is attributed to the

degeneration of motor neurons in the central nervous system. Although more than 90% of ALS cases are sporadic, certain common mutation variants, such as Hexnucleotide Repeat Expansion (HRE) in the first intron of C9 or f72 and mutations in SOD1 TARDBP (coding for TDP-43) and FUS have been identified as triggers for ALS to varying extents [26-31].

The utilization of iPSChas become pivotal in modelling ALS. A crucial aspect of iPSCapplication in modelling ALS involves direct differentiation into Spinal Motor Neurons (SMNs). Well-established protocols involve SMAD inhibition by small molecules, followed by a later direction toward caudal or ventral identity with induction signals [32-34]. Advanced screening and imaging techniques, such as Multi Electrode Arrays (MEA) have further facilitated precise measurement and assessment of parameters, including neuronal activity and connectivity throughout the reprogramming process. The pathology of ALS involves the restricted degeneration of specialized groups of neurons in the motor cortex and severely affects cortical interconnections. Hence, many studies have focused on inducing ALS in the cortical neurons [35-39]. Additionally, neuroinflammation plays a critical role in ALS pathology, and controlling neuroinflammation by redirecting the communication between motor neurons and glia is a promising therapeutic research area. Therefore, to model this mechanism, scientists have also developed a reprogramming protocol for iPSCinto astrocytes and microglial cells which play important roles in neuroinflammation. [40-45].

Traditionally, animal models have been extensively used in research uncovering cellular pathology in ALS, while overexpression of mutated human genes in animal models may lead to a strong phenotype that is not representative enough for real-life patient conditions. iPSC, with the ability to differentiate into various ALS-related neurons, can provide an alternative research platform to traditional rodent, Drosophila, yeast, and human brain tissue models and is significant in uncovering disease pathophysiology. iPSC-derived motor neurons harboring mutations in C9 or f72 SOD1 TARDBP or FUS have been extensively utilized to study molecular and cellular interaction modifications in ALS. Comparative studies have revealed shared pathological phenotypes across diverse hiPSCALS models, including mitochondrial dysfunction and oxidative stress, while some phenotypes, such as nucleocytoplasmic transport defects, are model-specific [46-61]. Beyond the cell-autonomous mechanism, interactions between different types of cells in the nervous system add another layer of complexity to unravel the ALS mechanism. Recently, some studies have shown that iPSCcan differentiate into a combination of different cell types in the CNS, which provides a promising tool for studying cell-cell interactions, such as iPSC-derived astrocytes/motor neuron coculture Notably, newly developed techniques, such as the on-chip neuromuscular model and assembloid model (the combination of multiple organoids) provide deeper insight into cell-cell and cell-environment crosstalk, rising the iPSC-based ALS modelling to a higher level [62,63] .

In addition to neurodegenerative diseases, patient-derived iPSChave been widely applied in modelling neuropsychiatric diseases and play an important role in uncovering cellular phenotypes, including synaptic dysregulation in schizophrenia and altered calcium ion signalling in autism [94-99].

Drug and Therapeutic Development

iPSC-derived cell products can be utilized to delineate disease pathophysiology and etiology by offering a robust disease model capable of recapitulating the intricate processes involved in disease initiation and progression. Within the realm of pharmaceutical research and development, these cell products play a crucial role in assessing the toxicity and potency of drugs, thereby providing insights into the underlying molecular pathways. Furthermore, iPSC-derived cell products have immense

potential for advancing cell therapy and personalized medicine, representing pivotal directions in the trajectory of future human healthcare initiatives.

Neurotoxicity Test with iPSC-derived cell culture

Neurotoxicity testing is a pivotal step in the compound testing process of drug discovery; however, it is expensive and time-consuming. Traditional neurotoxicity assessments using animal models have been further encumbered by ethical concerns. Pioneering studies have embraced the utilization of iPSC-derived neuronal models that have no ethical issues and can faithfully recapitulate human neuronal physiology for in vitro neurotoxicity evaluations involving neurogenic compounds and neurotoxicants. Subsequent investigations have underscored the efficacy of the hiPSC-derived neuron co-culture model as a prioritized tool for neurotoxicity testing, demonstrating its superiority in yielding pertinent results [100]. hiPSC-derived culture systems have been subsequently employed to model and assess neurotoxicity induced by various factors, including amyloid-β antidepressant paroxetine oxygen-glucose deprivation pesticide-related compounds chemotherapy and many other environmental chemicals [101-106].

Drug Discovery and Potency Assessment with iPSC-derived Organoids

Traditional homogeneous 2D cultures and spheroids are inherently limited in their ability to visualize and assess the intricate molecular processes underlying disease progression or treatment responses owing to the absence of interorgan communication. To address this limitation, an innovative stem cell-derived technique, known as organoids, has emerged in the past decade. Organoids, which are 3D multicellular cultures that are artificially generated from stem cells, represent a transformative approach. Despite the complexity of the brain, cerebral organoids have proven instrumental in scientific research concerning brain structure, disease and development, neurotoxicity, and viral infections under in vitro conditions [100-110]. The advent of neural organoids not only facilitates scientific research, but also fosters collaboration between academia and industry. Traditionally reliant on animal models, the drug discovery process has encountered challenges due to physiological differences between animals and humans, resulting in a barrier between pre-clinical and clinical assessments and contributing to late-stage drug failures. In contrast to traditional 2D cultures, neural organoids exhibit near-physiological cellular composition and maintain genome stabilization during development, making them promising candidates for high-throughput screening and drug discovery. In the realm of Alzheimer's disease drug development, iPSC-derived neurons were employed to identify compounds inhibiting or downregulating amyloid-β secretion Various companies have used cerebral organoids in the drug discovery process for Rett syndrome , schizophrenia and epilepsy [111-114]. Cerebral organoids also play a pivotal role in neurogenomics [115]. One study demonstrated that the gene expression programs of the fetal neocortex are highly similar to human cerebral organoids, with just a few gene exceptions, by utilizing single-cell RNA sequencing, which provides evidence that cerebral organoids are a robust tool for studying human brain development deficiency [116]. With the increasing availability of single-cell transcriptomic datasets, detailed comparisons between organoids and their primary counterparts can be performed [117]. Beyond the transcriptome, cerebral organoids faithfully recapitulated epigenomic changes observed in the primary human brain. Including alterations in chromatin accessibility throughout neurodevelopment, also occurs in brain organoids [118-121]. Generating brain organoids from patient-derived iPSCs replicates the genetic makeup of the disease, offering a valuable tool for investigating the relationship between risk variants and cellular phenotypes in disease pathology (Figure 3).

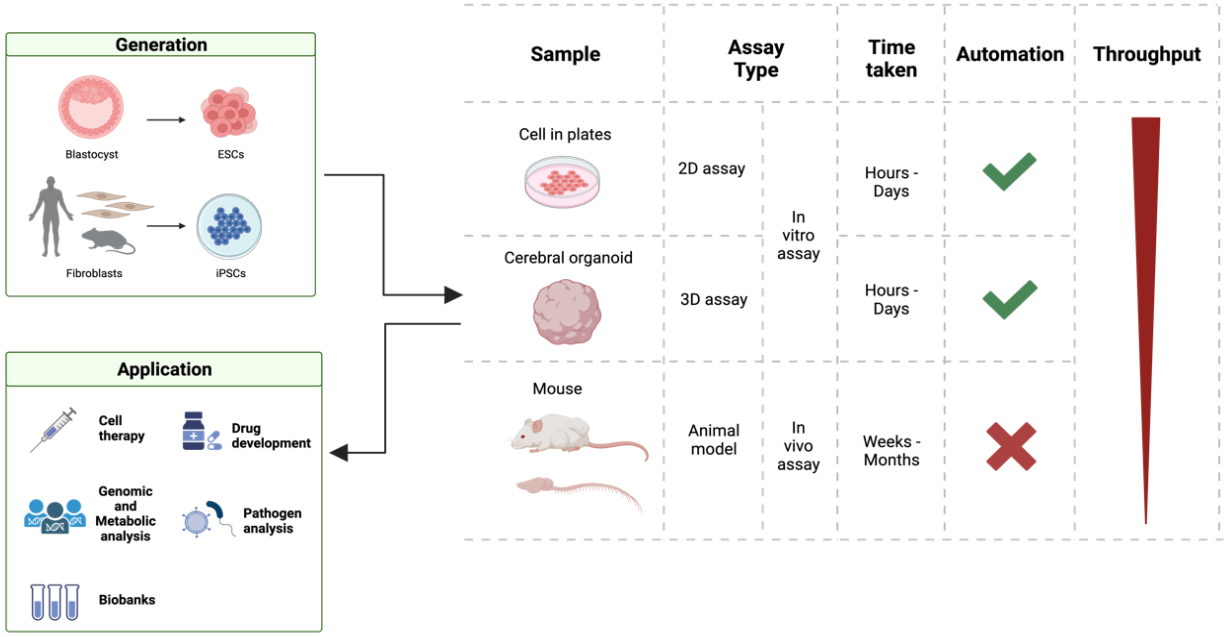


Figure 3 Summary of the generation process and application of cerebral organoids with a comparison table between in vivo and in vitro assay types

Cell Therapy with Ipsc-Derived Neuron

Direct use of iPSCs in cell therapy, including neurogenesis and cellular transplantation, has been well developed and has entered clinical trials for various neurodegenerative diseases (e.g., Parkinson's disease, macular degeneration) and neurological injury (e.g., spinal cord injury).

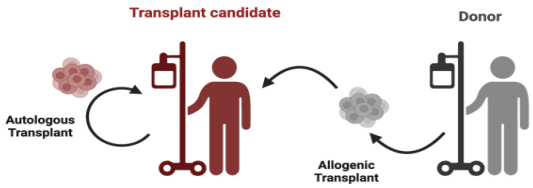
Utilizing Dopaminergic Progenitors (DAPs) derived from human iPSCfor treating PD is a relatively mature and successful approach for cell therapy. High-quality DAPs can be generated without undifferentiated iPSCs, early neural stem cells, transformed cells (based on cellular genome screening and comparison with the COSMIC Census and cancer-related gene list), or cells with abnormal epigenetic modifications detected after the differentiation process. Furthermore, based on single-cell RT-qPCR analysis, no gene expression difference was observed relative to the DA differentiation process, which confirms the reproducibility of cell manufacturing. Therefore, the safety and efficacy of clinical-grade cell products have been confirmed, and this iPSC-derived DAP cell therapy is currently in phase 1/2 clinical trials [122].

iPSC also represent a potential breakthrough in the treatment of Spinal Cord Injury (SCI). Given the crucial role of the spinal cord in facilitating signal transmission between the brain and peripheral body parts, any injury to this delicate structure can result in severe impairment of both mobility and sensory functions. While previous studies have demonstrated the efficacy of Neural Progenitor Cell (NPC) transplantation in the treatment of SCI in animal models, the ethical issues associated with the use of fetal neural stem cells or hESCs have impeded the translation of these findings to clinical applications. Therefore, hiPSC-induced NPCs represent a novel avenue for SCI treatment. Studies have revealed that hiPSC-NPCs successfully undergo neuronal differentiation, establish synaptic connections, and enhance axonal growth and angiogenesis in rodent SCI models without generating tumors within three months of transplantation [123]. Subsequent experiments with non-human primates corroborated these results. Furthermore, the development of medications capable of downregulating neuroinflammation has also demonstrated an augmentation in the therapeutic effectiveness of iPSC-NPC transplantation in

clinical settings. Several investigations have underscored the potential of hiPSC-NPCs in mitigating secondary damage associated with SCI. These cells exhibit the ability to reduce the levels of pro-inflammatory cytokines and form glial or fibrotic scars after SCI damage [124].

Notably, there are two main approaches to iPSC-based cell transplantation; autologous and allogeneic (Figure 4). Allogenic cell therapy is based on using iPSCsourced from someone other than the patient. Autologous therapy utilizes cell products derived from reprogramming of the patient's own cells. The first case of human autologous pluripotent stem cell transplantation utilized retinal pigmentary epithelial cells derived from iPSCfor treating age-related macular degeneration without being given an immune suppressant [125]. The result was promising, with no adverse effects after more than one year of therapy and the visual acuity of the patient was stabilized. However, in the second clinical trial, three single nucleotide mutations were found during safety testing of the patient's iPSC-derived cells that were not present in their original cell sources, and more importantly, one of the three mutations was considered to be a cancer-associated mutation [126]. This has raised concerns about the safety issues associated with autologous iPSC transplantation.

Autologous therapy, while maintaining the potential to mitigate or eliminate the need for immunosuppression, encounters distinct challenges in comparison to allogeneic transplantation when translated into practical applications. First, the genetic constitution and immunogenicity of the cell products may undergo alterations throughout the reprogramming process. Second, in the context of neural transplantation, cells are transplanted into an immunologically privileged site, obviating the imperative for immunosuppression and thereby attenuating the inherent advantage of autologous transplantation. Moreover, allogeneic therapy benefits from a protracted development timeline, allowing for rigorous cellular-based characterization during preclinical assessments, and the resulting iPSC-derived cell products can be transplanted into multiple recipients over an extended period. Conversely, the personalized nature of autologous transplantation necessitates a condensed development timeline for each transplantation within a clinically relevant timeframe. This temporal constraint precludes the completion of comprehensive long-term animal safety studies, rendering autologous transplantation fraught with relatively higher risks, diminished scalability, and a heightened requirement for regulatory and assessment approaches. Additionally, the scenario of patients harboring Mendelian diseases poses a nuanced challenge because utilizing cells with mutations for reprogramming may yield suboptimal outcomes [127]. In response to these challenges, a novel therapeutic approach that combines the advantages of autologous therapy with a reduced need for immunosuppressants and the economics of allogeneic therapy has emerged. This innovative strategy involves the implementation of Human Leukocyte Antigen (HLA) subtype matching through the establishment of iPSC "haplobanking." The iPSChaplobank was set by banking iPSCcell lines deliberately selected to be homozygous for diverse HLA haplotypes. This enables the derivation and selection of therapeutic products tailored to the immunological conditions of individual patients. This method facilitates the customization of cell lines matching the HLA of the recipient, enhancing the efficiency of generating substantial quantities of quality-controlled transplantable cells. Moreover, this approach, which resembles an off-the-shelf product, holds particular promise for addressing critical subacute conditions such as spinal cord injury [128].



	Need for Immunosuppression	Potential mutation in cell resources	Risk of therapy	Scalability	Time available for therapy development
Autologous transplant	Theoretically no, but immunogenicity may change throughout the reprogramming process	Yes			
Allogenic transplant	Yes	No			

Figure 4 Summary and comparison table of autologous and allogeneic stem cell transplantation

Challenges and Opportunities

Despite the considerable promise of iPSCtechnology as a prospective tool for disease treatment and modelling, and its subsequent application in clinical settings, numerous impediments persist in the translation of iPSC-based cell products into widespread clinical utility.

Tumorigenicity: The inherent tumorigenicity associated with this approach is a significant impediment in iPSCtherapy. Tumorigenicity can arise from several factors, including the persistence of residual PSCs or progeny cells that retain their proliferative capabilities. The involvement of reprogramming factors and genetic alterations occurs during in vitro cell culturing processes. Even minute quantities of inaccurately patterned residual iPSCs in the final cell product can induce teratoma formation, as observed by the emergence of neural rosettes during the neural differentiation process involving hESCs or hiPSCs [129].

Efforts have been made to avoid the formation of teratomas, including the development of an efficient and accurate in vitro-directed differentiation protocol or detailed iPSC-derived cell product purification with antibodies [130]. Beyond the persistence of residual PSCs, the proliferative nature of iPSC-derived cell products can independently contribute to tumor formation, as evidenced by hiPSC-based interventions for spinal cord injury in non-human primate models [131]. Reprogramming is another source of tumorigenicity. One of the four reprogramming factors of iPSC, c-Myc, is one of the most frequently mutated genes in human cancers and always acts as a mutation driver. Besides, apart from the original four reprogramming factors, some other factors may increase reprogramming efficiency, including EBNA1 or the dominant negative mutant of p53, which are also believed to be closely related to cancer development [132]. These genetic changes during the culture process add another layer of complexity to the challenge of ensuring genomic integrity of iPSC-derived cell products for clinical applications.

Immunogenicity: Another important concern in iPSCtherapy is the immunogenicity caused by cell transplantation. PSC transplantation holds promise as a rejection-free cell therapy, and the prevailing clinical preference is currently inclined towards allografts with immunosuppressive interventions, given the considerations of time and economic feasibility. Despite immune-privileged sites, such as the Central Nervous System (CNS) and eyes exhibiting a reduced requirement for immunosuppression, factors such as aging, trauma, or diseases can compromise this immune privilege. At non-immune-privileged sites, lifelong immunosuppression may be imperative for successful cell transplantation.

Strategies have been developed to address immunological rejection, including HLA matching, through the establishment of a haplotype bank of iPSCcell lines and the HLA cloaking approach. Taylor. established an iPSCbank based on 150 selected homozygous HLA-type volunteers, which can cover 93% of the entire UK population with a minimal requirement for immunosuppression [133]. However, conflicting results have emerged regarding the efficacy of HLA matching in CNS cell transplantations. Primate studies have produced contradictory outcomes, with one group demonstrating the effectiveness of

HLA matching in reducing immune rejection and increasing engraftment success [134]. Conversely, another study reported that HLA matching failed to prevent long-term rejection of iPSC-derived neural engraftment [135].

The HLA cloaking approach is a relatively new method facilitated by the advancement of gene editing technology such as CRISPR, which involves manipulating the gene encoding HLA Class I and Class II or modifying cells to express immunosuppressive molecules such as PD-L1 and CTLA4-Ig. This enables iPSC-derived cells to evade immune detection and function as universal donor cells. However, the safety risks associated with this cloaking method necessitate thorough discussion and examination, including concerns about potential immune evasion by malignant-transformed and viral-infected transplanted cells [136]. One proposed solution involves equipping these universal cells with an inducible caspase-9 suicide gene system that automatically triggers apoptosis when the cell is infected or becomes tumorigenic [137].

Heterogeneity: Each iPSCline exhibits distinct characteristics, encompassing differences in the growth curve, morphology, differentiation efficiency, and gene expression. This intrinsic genetic heterogeneity is believed to be influenced by background mutations in parental cells [138]. Manifests in varying expression levels of lineage-specific genes in different iPSCcell lines. This diversity renders certain cell lines more suitable for neurogenesis, whereas others may excel in cardiomyocyte regeneration. Additionally, variations in differentiation ability and efficiency are evident, with some iPSCcell lines displaying deficiencies compared with their counterparts. For instance, while the majority of iPSCs exhibit comparable neural differentiation efficiency to Embryonic Stem Cells (ESCs), certain lines may only achieve 80% differentiation efficiency, with residual undifferentiated cells posing an elevated risk of teratoma formation in the brain [139]. The primary contributor to iPSCheterogeneity is the genetic background variation in the donor. A comprehensive study involving genotyping and phenotyping of 711 iPSCcell lines derived from 301 individuals revealed that 5%-46% of the variation arises from inter-individual differences [140]. In addition, even though the majority of epigenetic modifications are erased during the reprogramming process, the retaining modifications still account for the inter- and intra-individual iPSCline variability [141]. Other factors influencing iPSCline heterogeneity include UV-induced somatic mutations [142] and discrepancies in cell culture and handling practices, such as passage number and culture variability [143-145].

Strategies to mitigate heterogeneity involve the establishment of control iPSCcell lines that are shared among different research communities, allowing for effective cross-experiment comparisons. Standardization of iPSCreprogramming and differentiation protocols coupled with the implementation of a functional quality control system can decrease intra-clone variation. Utilizing advanced computational technologies such as single-cell RNA sequencing facilitates the identification and clustering of heterogeneous cell populations. Notably, genetic heterogeneity in iPSCs may not always be disadvantageous. Single-cell analyses of cell types and intra-culture heterogeneity may reveal unique developmental phenotypes that are influenced by genetic variants. For instance, in iPSCs derived from patients with metachromatic leukodystrophy, disease-causing mutations favor the maintenance of immature oligodendrocytes with weakened neuronal support capabilities, ultimately leading to neuronal death [146].

Recapitulating Polygenic and Sporadic Diseases

As highlighted in the preceding sections, most neurological disorders exhibit a sporadic nature, characterized by diverse pathologies and symptoms, thereby posing a challenge for patient-derived hiPSCs to adequately represent the varied presentations of these diseases. This inherent diversity in patient-derived hiPSCs can introduce bias into modelling outcomes. However, recent advancements in pooled technologies, exemplified by the "village-in-a-dish" model, offer a potential solution

to this challenge. This innovative model involves the amalgamation of cells from up to 100 iPSCdonors that are co-cultured in a shared environment and subsequently evaluated for phenotypes. Following the identification of a specific phenotype of interest, DNA within the collective cell population was sequenced, and a systematic computational analysis was employed to discern the proportion of cells from each donor contributing to the observed phenotypic variation. This information is then mapped back to the underlying genomic variation, effectively reducing inter-line variation and enhancing the representativeness of the model [147]. Furthermore, transformative gene-editing technologies such as CRISPR-Cas have been seamlessly integrated into culture systems. This technological adaptation enables the eradication of genetic background variations, thereby facilitating the generation of isogenic iPSCcell lines. Such isogenic lines are instrumental in studying the intricate relationship between diseases and specific mutations, allowing for a more controlled and focused exploration of pathogenic mechanisms [148].

Lack of Clinical History Data From iPSC Donor

The persistent absence of comprehensive clinical history in iPSCdonor patients is a notable constraint that significantly contributes to the observed genetic background variations among iPSCcell lines or clones. Comprehensive clinical information plays a pivotal role in elucidating iPSCheterogeneity, offering critical insights into the underlying genetic factors. Consequently, the limitations imposed by the absence or incompleteness of these clinical data hinder the accurate interpretation of the experimental results, impeding a comprehensive understanding of the observed outcomes. Addressing this challenge involves the establishment of iPSCbanks characterized by rigorous standardization of cell source selection, iPSCproduction procedures, and cell storage and distribution processes. Such systematic standardization provides a substantial opportunity for researchers to access more detailed information about cell products, facilitating a more precise and informed analysis of iPSC-derived materials. The development of iPSCbanks governed by stringent protocols has emerged as a valuable approach for enhancing the reliability and depth of investigations into iPSCheterogeneity and associated genetic factors [149].

Scalability

Another significant constraint in hiPSCexperiments lies in the scalability of traditional cell culture and reprogramming protocols, which poses challenges in achieving large-scale production of cells of interest with both high reproducibility and efficiency. However, recent technological advancements such as automated and high-throughput cell culture systems, high-content image-based assays and Probe-Based Imaging For Sequential Multiplexing (PRISM)(152) have substantially enhanced experimental scalability. These technologies enable the expansion of the sample sizes tested while maintaining precision at the single-cell level, thereby broadening the potential applications of iPSC.

The synergy between traditional iPSCtechniques and bioengineering approaches, including organoids organ-on-chip and 3D printing further extends the potential applications of iPSCto both clinical and academic settings. An intriguing avenue of exploration involves the application of CRISPR interference (CRISPR-i) to iPSCtechnology. CRISPR-i employs Cas9 protein fused with a transcriptional repressor domain that lacks DNA-cutting activity but is capable of suppressing gene expression. Using this technology, in conjunction with single-cell RNA-seq screening and arrayed high-content screening, scientists can identify genes essential for or enhance neuronal survival and development in hiPSC-derived neurons. This approach provides a robust platform for systematically investigating the health and disease states of neurons in iPSCmodels [150-157].

Late-Onset Disease Modelling

A significant impediment in using iPSCs for modelling neurological diseases arises from the reprogramming process, in which the epigenetic characteristics of cells are reset, leading to the regression of age-related cellular features, including mitochondrial function, telomere length, and proteostasis, to embryonic states. Consequently, iPSC-derived neurons lack ideal characteristics for modelling age-related late-onset neurological diseases [158,159]. Various strategies have been devised to overcome this limitation. For instance, the induction of neurons with aging-like features from iPSCs has been pursued through telomere manipulation or pregerin induction [160,161]. A more direct approach is the implementation of direct induced Neuron (iN) technology, which is capable of maintaining the epigenetic signature of donor cells, offering a promising tool for studying late-onset neurological diseases. This relatively new technology was first attempted by the Wernig group in 2010. They successfully induced mouse fibroblasts to differentiate into neuronal cells by expressing three vital transcription factors: BRN2, ASCL1, and MYT1L [162]. Direct reprogramming involves the activation of neuronal-fate-determining genes through microRNAs, chemical modulation of key signaling pathways, gene overexpression via viral vectors, or a combination of these strategies. iN neurons have been widely used in neuroscience research, with applications ranging from the modelling of functional DA neurons and MN for studying PD and ALS, respectively. Recently, iNs mirroring the pathophysiological features of sAD have also been generated [163-165]. However, iN technology exhibits limitations, particularly in terms of lower reproducibility and efficiency, compared to traditional iPSCtechnology. The absence of a highly expandable reprogramming intermediate stage in the induction process and the post mitotic state of the produced neurons hamper their proliferative capacity, limiting the scale of iN generation to fibroblast proliferation. Additionally, the conversion rate of iNs is relatively low, ranging from 5%-30% depending on the source cell type. These challenges constrain the contribution of iNs to drug screening and other processes that require a large number of reproducible cells. In contrast, iPSCs, once derived, possess the capability of infinite expansion, offering a vast resource for generating a large number of neurons for subsequent research endeavors.

CONCLUSION

We examined the contemporary applications of iPSCtechnology within the realm of neuroscience, specifically focusing on its transformative role in addressing neurological diseases. The historical limitations imposed by species barriers in animal models and constraints associated with human patient samples have impeded the progress of disease research in neuroscience. iPSC-induced neurons have emerged as a promising paradigm for modelling various neurodegenerative diseases and traumatic conditions. These iPSC-derived neurons effectively encapsulate the disease-associated genetic background and faithfully replicate the cellular pathology of neurological disorders, thus enabling a profound understanding of their etiology and progression.

Leveraging the ethical advantages and perpetual replicative potential of iPSC-derived cells, researchers have extensively employed them in drug and therapeutic development. Innovative technologies, such as cerebral organoids, have introduced 3D structures that mimic organ architecture, fostering a nuanced representation of cellular interactions within tissues and broadening the scope of iPSCapplications. Furthermore, autologous and allogeneic cell transplantation of iPSC-derived neurons holds promise for the treatment of neurodegenerative diseases.

However, despite these advancements, the iPSCmodel faces significant challenges that hinder its widespread clinical application. Concerns persist regarding tumorigenicity, immune rejection, and inherent heterogeneity of iPSC-derived cells.

Additionally, as a relatively recent technology, standardized iPSCreprogramming and induction systems have yet to be fully established. Questions regarding the representativeness of a specific patient's iPSC-derived cell line for modelling sporadic neurological disorders, the establishment of a universal standard for iPSC-related research, and the mitigation of tumor emergence and immune rejection post-cell transplantation remain critical areas that require further investigation.

Notwithstanding these challenges, significant progress has been made in this field. Emerging strategies and technologies, such as the iN approach, pooled technology, and genetically modified iPSCs, is promising avenues for overcoming the existing limitations. Interdisciplinary collaborations, particularly between iPSCtechnology and fields such as bioengineering and gene editing techniques, have yielded innovative approaches such as organ-on-chip and tissue engineering. These advances present new possibilities for advancing our understanding and treatment of neurological diseases.

As the field progresses, the continuous exploration of novel strategies, interdisciplinary collaborations, and integration of emerging technologies will undoubtedly contribute to the ongoing evolution of iPSCapplications in neuroscience and the broader biomedical landscape.

DECLARATIONS

Ethical Approval

Not applicable

Consent to Participate

I read and understood the information provided above. I voluntarily agreed to participate in this study and consented to use my responses for research purposes.

Consent to Publish

I read and understood the information provided above regarding the potential publication of the research findings. I voluntarily consented to the publication of anonymized data from my participation in this study.

Authors Contributions

Xinran Zhang conceived and designed the study, collected and analyzed the data, and drafted the manuscript. The authors have read and approved the final manuscript.

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Competing Interests

The authors declare that they have no conflicts of interest. No funding agency or external organization played any role in the study design, data collection, analysis, interpretation, or manuscript preparation.

Availability of Data and Materials

Not applicable

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