Neural stem cells for Parkinson's disease management: Challenges, nanobased support, and prospects

Tuba Oz, Ajeet Kaushik, Małgorzata Kujawska

Tuba Oz, Małgorzata Kujawska, Department of Toxicology, Poznan University of Medical Sciences, Poznan 60-631, Poland

Ajeet Kaushik, NanoBioTech Laboratory, Health System Engineering, Department of Environmental Engineering, Florida Polytechnic University, Lakeland, FL 33805, United States

Ajeet Kaushik, School of Engineering, University of Petroleum and Energy Studies, Dehradun 248007, India

Corresponding author: Małgorzata Kujawska, MSc, PhD, DSc, Professor, Department of Toxicology, Poznan University of Medical Sciences, Dojazd 30, Poznan 60-631, Poland. kujawska@ump.edu.pl

Abstract

Parkinson's disease (PD), characterized by loss of nigrostriatal dopaminergic neurons, is one of the most predominant neurodegenerative diseases affecting the elderly population worldwide. The concept of stem cell therapy in managing neurodegenerative diseases has evolved over the years and has recently rapidly progressed. Neural stem cells (NSCs) have a few key features, including self-renewal, proliferation, and multipotency, which make them a promising agent targeting neurodegeneration. It is generally agreed that challenges for NSC-based therapy are present at every stage of the transplantation process, including preoperative cell preparation and quality control, perioperative procedures, and postoperative graft preservation, adherence, and overall therapy success. In this review, we provided a comprehensive, careful, and critical discussion of experimental and clinical data alongside the pros and cons of NSC-based therapy in PD. Given the state-of-the-art accomplishments of stem cell therapy, gene therapy, and nanotechnology, we shed light on the perspective of complementing the advantages of each process by developing nano-stem cell therapy, which is currently a research hotspot. Although various obstacles and challenges remain, nano-stem cell therapy holds promise to cure PD, however, continuous improvement and development from the stage of laboratory experiments to the clinical application are necessary.

Key Words: Parkinson's disease; Synuclein; Neural stem cells; Nanomaterials; Nano-stem cell therapy

Core tip: Neural stem cell (NSC) transplantation is a groundbreaking therapy with therapeutic effects for many neurodegenerative diseases. Parkinson's disease (PD) is the second most common neurodegenerative movement disorder. The ability of NSCs for neural differentiation has been suggested as an effective mechanism in PD subjected to cell transplantation. Here, the potential therapeutic mechanisms, challenges, nanobased support, and manifestations of NSCs in PD are discussed, along with examples of preclinical and clinical studies.

INTRODUCTION

Parkinson's disease (PD), with a global prevalence of > 6 million, is the second most common neurodegenerative movement disorder. Currently, no disease-modifying cure is available, and occurrence of PD has been projected to double over the next generation[1]. PD is an age-related disorder affecting about 5% of the population by the age of over 85 years.

The primary pathological property of PD is the presence of abnormal α-synuclein (ASN) in the form of neural inclusions called Lewy bodies, whose toxic effect is hypothesized to contribute to progressive degeneration and cell loss [2,3]. Dopaminergic (DAergic) neurons in the substantia nigra pars compacta specifically undergo degeneration leading to dopamine (DA) and several other biochemical deficits in the nigrostriatal system, resulting in the development of motor symptoms, including bradykinesia, resting tremor, muscular rigidity, and postural instability[4]. Moreover, loss of other types of neurons and the presence of Lewy pathology in many parts of the central, enteric, and autonomic nervous systems have been demonstrated that probably contribute to non-motor manifestations, such as autonomic dysfunction, olfactory impairment, and mood, cognitive or sleep disturbances present in PD patients[5,6].

Dopamine replacement therapy (DRT) is the gold standard treatment for alleviating PD symptoms. However, as the disease progresses, compensatory mechanisms for denervation affect the duration and delay the alleviating effect of DRT in a nonlinear manner[7]. Surgical procedures are also used, including stereotactic ablations or deep brain stimulation. Nevertheless, these approaches only partially manage the severe motor symptoms and alleviate DAergic desensitization supporting DRT in advanced PD patients[8,9]. Aggregation of ASN, besides the gain-of-toxic function, can also result in loss of function, thereby decreasing its functions[10]. Since ASN exerts physiological activity towards newly formed neurons by promoting the development and maturation of dendrites and the spine, depending on the expression level [11], the intraneuronal inclusions of ASN contribute to the PD pathogenesis by also affecting neurogenesis[12]. Therefore, stimulation of endogenous neurogenesis or cell-replacement therapy is considered a therapeutical option against PD neurodegeneration[13]. Accordingly, strategies aiming at rebuilding the pathway and reshaping the brain have been developing[14].

Endogenous neural stem cells (NSCs) exist throughout life and are found in specific areas of the human brain. NSCs exhibiting abilities to self-renew and differentiate into neurons, astrocytes, or oligodendroglia are responsible for restoring brain function under normal circumstances. The regeneration of DAergic neurons from stem cells is considered an alternative treatment for PD[15].

In the early phase of preclinical and clinical studies, foetal ventral midbrain tissues were grafted into the striatum of rats[16,17] and PD patients[18-23]. The foetal DAergic neuron transplants survived, released DA, and improved behavioural deficits in animal models of PD[16,17] and individuals[20,21]. However, apart from these benefits, in some patients, dyskinesia developed[22,23]. Moreover, independent postmortem examinations revealed that the healthy neurons grafted into the brains of PD patients acquired Lewy bodies several years after transplantation[18,19] due to a prion-like mechanism with permissive templating of synucleinopathy progression[1,6]. In this context, DA neuron-based transplant approaches are not curative; nevertheless, they offer a competitive therapeutic effect[5]. The rapid development of cell- and molecular-based research opens new perspectives for cell therapy approaches in managing PD [5]. During the last decade, cumulative research on using different types of stem cells as starting material supports the potential of stem-cell-based DAergic neuron replacement therapy for PD[5]. The considered candidates are NSCs, embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and mesenchymal stem cells (MSCs)[24]. Importantly, translation into the clinic of the stem cells transplantation approach requires avoidance of adverse immunological response and proliferation and related risk of tumorigenesis[5].

This review focuses on the therapeutic potential of NSCs in PD. Accordingly, we give an overview of their use in experimental studies and clinical trials and discuss challenges related to their application, alongside the pros and cons of NSC-based therapy in PD. Given the state-of-the-art accomplishments of stem cell therapy, gene therapy, and nanotechnology, we shed light on the perspective of complementing the advantages of each process by developing nano-stem cell therapy, also using genetically engineered NSCs.

EMERGENCE OF NSCS IN ADULT BRAIN AND ALTERNATIVE SOURCES OF NSCS

NSCs are an important central nervous system (CNS) element. NSCs exist in the embryonic brain and adult nervous system during human life and are found in two specific brain regions (Figure 1A). The subgranular region of the dentate gyrus and the lateral wall of the subventricular zone both contain a significant number of adult endogenous NSCs[25]. These cells have a few key features, including self-renewal, proliferation, and multipotency, which make them a major component in the CNS, maintaining the cell pool of nervous tissues[15]. NSCs can differentiate into neurons, astrocytes, or oligodendrocytes and may replace the loss of DAergic neurons in PD[13]. Moreover, NSCs can encourage endogenous repair mechanisms as they can migrate near the site of damage to increase neuroblasts and promote tissue repair. NSCs can have immunomodulatory effects such as cytokine, chemokines, and chemokine receptors secretion and T cell proliferation inhibition[26,27] (Figure 1B). Neurotransmitters, neuropeptides, cytokines, metabolites, extracellular matrix proteins, and accessory cells control NSCs proliferation[13].

The alternative sources of NSCs are foetal and adult nervous system tissues, umbilical cord blood, bone marrow, peripheral blood, amniotic fluid, Wharton jelly, skin- or adipose-derived MSCs, or iPSCs[28] (Figure 1A). In the transplantation procedure, these stem cells are either *in vitro* pre-differentiated or, according to the concept that signals in the injured tissue will recruit the stem cells and induce their differentiation *in situ*, NSCs are applied directly in the undifferentiated state[28]. Recently, trans-differentiation, especially chemically induced, whereby somatic cells are reprogrammed into patient-specific neuronal cells by skipping an intermediate proliferative pluripotent stem cell stage, offers perspectives on alternative autologous cell therapeutic strategies for PD[29]. By producing the desired type of CNS cells, these sources have all been used to demonstrate their potential for treating various neurodegenerative diseases[30].

EXPERIMENTAL APPROACHES TO NSC TRANSPLANTATION

Several preclinical studies have shown that different types of human NSCs, after their transplantation, could migrate, survive, and proliferate in specific brain sites. Immunohistochemical examinations support evidence that the transplanted human NSCs could transform into functional neurons and integrate into neural circuits[31]. In *in vivo* studies, it has been demonstrated that NSCs transplantation was an efficient therapeutic method for PD (Table 1). Yang *et al*[32] have shown that the clonal line C17.2 of NSCs grown *in vitro* under control conditions and transplanted into intact or 6-hydroxydopamine (6-OHDA)-lesioned rats spontaneously developed into neuronal-like cells, expressed traits of a DA phenotype, and integrated into the host brain[32]. Svendsen *et al*[33] have demonstrated that expanded populations of human CNS progenitor cells maintained in a proliferative state in culture and can migrate and differentiate into both neurons and astrocytes following intracerebral grafting into the striatum of adult rats with unilateral DAergic lesions[33]. NSCs, secreting *in vitro* nerve growth factor and neurotrophin-3, engrafted into the 6-OHDA lesioned striatum of rats, have been reported to survive and partially differentiate into tyrosine hydroxylase (TH)-positive cells. A significant rotational behaviour improvement accompanied these effects[33].

The ability of NSCs for neural differentiation and to exert a trophic effect by secreting nerve growth factor and neurotrophin-3 on dying DA neurons in the striatum has been suggested as an effective mechanism against behavioural deficits in PD rats subjected to cell transplantation[34]. A high-throughput quantitative proteomic study revealed that the protein profile of the subventricular zone severely distorted in mice by 6-OHDA injection was normalized and striatal astrocytes were activated after human NSC transplantation accompanied by an increase in neurotrophic factors. In addition, the grafted mice exhibited improved behavioural performance. The changes in the proteome have been suggested to result from the stimulation of host nigral DAergic neurons and their nigrostriatal projections rather than their replacement[35]. To increase trophic factor secretion, genetically engineered human NSCs were developed[36]. Stereotaxic transplantation of human NSCs (HB1.F3 clone) secreting stem cell factor into the 6-OHDA-lesioned striatum of rats has been demonstrated to result in functional improvements and ameliorated Parkinsonian behavioural symptoms. It was accompanied by the activation of endogenous neurogenesis in the subventricular zone, alongside the preservation of TH-positive cells of the nigrostriatal pathway[37]. Similarly, human olfactory bulb NSCs genetically engineered to express human nerve growth factor ameliorated the cognitive deficits associated with 6-OHDA-induced lesions in PD model rats. Transplanted cells exhibiting enhanced survival and differentiation rate migrated to damaged areas to promote repair or neuroprotection through cell replacement, integration, and/or neuroprotection[38]. Foetus-derived human NSCs (hVM1 clone 32) transplanted in their undifferentiated state protected against motor and nonmotor deficits in middle-aged parkinsonian mice, although degeneration of the nigrostriatal pathway was not significantly improved. Thus, the multifactoriality of NSCs was suggested[39]. Yoon *et al*[40] have reported that although the human-brain-derived NSCs injected into the striatum of rats with the 6-OHDA-induced lesion of the nigrostriatal pathway differentiated into DAergic neurons, no functional or behavioural improvements were observed[40]. Transplantation of human-brain-derived NSCs in the form of cell aggregates with a pre-established cell network into the striatum of 6-OHDA-injected rats appeared to be more effective than single-cell transplantations. The transplanted cells not only survived but also exerted a therapeutic effect attributed to secreting DA[41].

Nonhuman primate models of PD represent the highest clinical translational value due to close-to-human similarities in physiology, anatomy, and immunology and are used to validate therapeutic strategies at preclinical stages[42]. Successful transplantation of undifferentiated human NSCs into the substantia nigra of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-challenged primates promoted homeostatic adjustments attributed to normalization of endogenous neuronal number-to-size ratios and protection of host nigrostriatal circuitry that resulted in behavioural improvement. In addition, the percentage of cells containing ASN inclusion dropped from 80% to 20% upon human NSC transplantation.

Table 1 Experimental studies on the therapeutic role of neural stem cells in Parkinson's disease models

Cells type	Model	Effects	Ref.
Undifferentiated NSCs line C17.2	6-OHDA rat model	Striatum deposition	Yang *et al*[32]
		↑ Expression of β-tubulin III, NSE, NeuN	
		↑ TH- and AADC-positive cells	
		↓ Motor behaviour deficits	
hCNSPCs	6-OHDA rat model	Striatum deposition	Svendsen *et al*[33]
		↑ Expression of β-tubulin III	
		↑ TH- and hGFAP-positive cells	
		↓ Motor behavior deficits	
hNSCs	6-OHDA mice model	Striatum deposition	Zuo *et al*[35]
		↓ IL-1 β, IL-2, and TNF-a	
		↑ IL-10	
		↓ Motor behavior deficits	
hfNSC line (HB1.F3)	6-OHDA rat model	Striatum deposition	Yasuhara *et al*[37]
		↑ Expression of nestin, HuD, β-tubulin III-positive, MAP2-positive, and NeuN positive and HuC negative	
		↑ TH-positive cells	
		↓ Motor behavior deficits	
hNFG-GFP-OBNSCs	6-OHDA rat model	Striatum deposition normal histoarchitecture of the striatum	Marei *et al*[38]
		↑ Striatal neurons	
		↓ Necrotic cells	
		↓ Motor behavior deficits	
human dental papilla-derived stem cells and hbNSCs	6-OHDA rat model	Striatum deposition	Yoon *et al*[40]
		↑ MAP2- positive cells	
		↑ TH- and GIRK2-positive cells	
		↔ No differences behavioral amelioration	
hbNSCs	6-OHDA rat model	Striatum deposition	Shin *et al*[41]
		↑ TH- and human β2 microglobulin-positive cells	
		↔ No differences behavioral amelioration	
hNSCs	MPTP-induced monkeys	Striatum deposition	Redmond *et al*[43]
		↑ TH- and DAT- positive cells	
		↑ DA level	
		↓ Motor behavior deficits	
hpNSCs	MPTP-induced monkeys	Striatum deposition	Gonzalez *et al*[44]
		↑ Striatal DA concentration, TH- positive cells, TH fiber innervation	
		↓ Behavior deficit	

6-OHDA: 6-hydroxydopamine; AADC: Aromatic L-amino decarboxylase; DA: Dopamine; DAergic: Dopaminergic; DAT: Dopamine transporter; GIRK2: G Protein-activated inward rectifier potassium channel 2; hNSCs: Human NSCs; hbNSCs: Human brain-derived NSCs; hfNSCs: Human fetal-derived NSCs; hGFAP: Human glial fibrillary acidic protein; hNFG-GFP-OBNSCs: Olfactory bulb neural stem cells (OBNSCs) genetically engineered to express hNGF and GFP; hpNSCs: Human parthenogenetic stem cell-derived NSCs; HuC: Very early neuronal marker; HuD: Postmitotic neuronal marker; MAP2:

Microtubule-associated protein 2; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NSE: Neuron-specific enolase; NeuN: Matured neuronal marker; NPCs: Neural progenitor cells; TH: Tyrosine hydroxylase; TNF-α: Tumor necrosis factor- α.
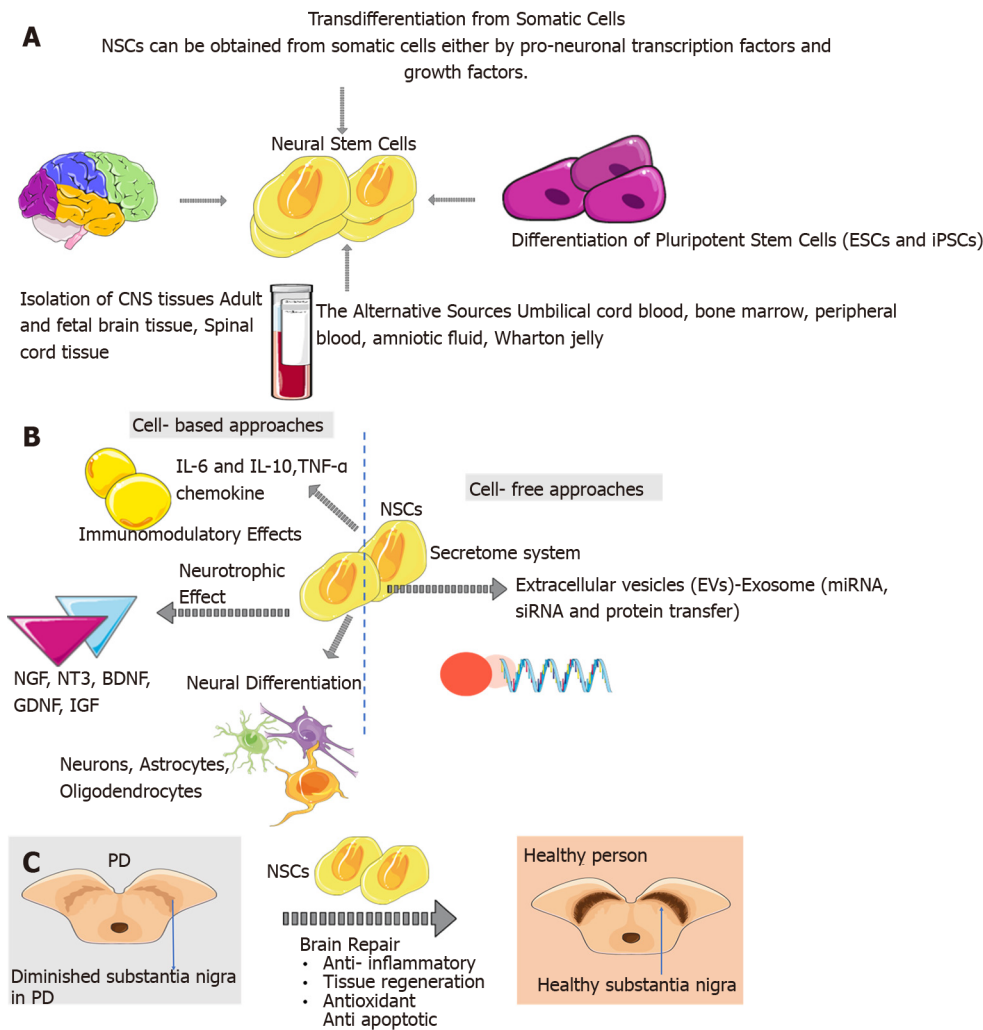
Figure 1 Emergence of neural stem cells in the adult brain and alternative sources of neural stem cells. A: The sources of neural stem cells (NSCs) are embryonic stem cells and induced pluripotent stem cells, adult/foetal brain tissue and spinal cord tissue. Also, NSCs can be obtained from somatic cells either by transcription factors and growth factors. The alternative sources of NSCs are foetal and adult nervous systems, umbilical cord blood, bone marrow, peripheral blood, amniotic fluid, Wharton jelly; B: NSCs can have immunomodulatory effects such as cytokine, chemokines, and chemokine receptors secretion and T cell proliferation inhibition. NSCs can have neurotrophic effects such as nerve growth factor, neurotrophin-3, brain-derived neurotrophic factor, and glial cell line-derived neurotrophic factor. NSCs can target secretome and paracrine effects *via* extracellular vesicles like exosomes. Also, NSCs play a role in neural differentiation; C: NSCs after their transplantation, could migrate, survive, and proliferate in specific brain sites. And NSCs play a role in brain repair mechanisms such as antiapoptotic, anti-inflammatory, and antioxidant. CNS: Central nervous system; BDNF: Brain-derived neurotrophic factor; ESCs: Embryonic stem cells; EVs: Extracellular vesicles; GDNF: Glial cell line-derived neurotrophic factor; iPSCs: Induced pluripotent stem cells; NGF: Nerve growth factor; NT-3: Neurotrophin-3; NSCs: Neural stem cells; TFs: Transcription factors; PD: Parkinson's disease; TGF: Transforming growth factor. Citation: The parts of the figures were drawn using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/Licenses/by/3.0/).

Importantly, no tumours, overgrowth, or inappropriate stem cell migration in the transplanted primates were observed [43]. Gonzalez *et al*[44] evaluated the safety and efficacy of two doses of human parthenogenetic stem-cell-derived NSCs injected in the striatum and substantia nigra of MPTP-lesioned monkeys with moderate to severe PD symptoms. They found that human parthenogenetic NSCs engrafts promoted behavioural recovery accompanied by raised striatal DA concentration, fibre innervation, and several DA neurons. Increased expression of genes and pathways downregulated in PD has been observed. Importantly, the 12 mo post-transplantation of human parthenogenetic NSCs was safe and well tolerated by the animals with no serious adverse events[44]. This safety aspect is significant as the potential for inappropriate stem cell migration and tumour formation must be minimized before cell transplantation[5].

Stem cell therapeutic strategies for PD not only deal with the direct replacement of damaged or lost cells but also target secretome and paracrine effects on neurotrophic and growth factors and endogenous neural precursor cell activity[45,46] (Figure 1B). The heterogeneous population of membrane-bound exosomes and extracellular vesicles (EVs) is a part of the

secretome system[45]. Nanosized EVs derived from human iPSC (hiPSC)-NSCs have been suggested as a safer alternative to NSCs. Upadhya *et al*[47] demonstrated that EVs exhibited similar neuroreparative properties as NSCs, and they could be administered noninvasively as an autologous or allogeneic off-the-shelf product. EVs comprising miRNAs and/or proteins promoting synaptogenesis, synaptic plasticity, and better cognitive function have been demonstrated to incorporate well into neurons, microglia, and astrocytes of rodents after intranasal treatment, resulting in enhancement of hippocampal neurogenesis and an anti-inflammatory effect[47]. The results hold the promise of using stem-/progenitor-cell-derived EVs, or EVs loaded with bioactive molecules such as DA, catalase, curcumin, and siRNAs, to alleviate parkinsonian symptoms[48]. Specifically, exosomes biologically active EVs, with high miR-133b, which are lacking in PD patients, can promote the growth of neurites[49]. Lee *et al*[50] have demonstrated that NSC-derived EVs *via* a paracrine signalling-based mechanism exerted antioxidant, antiapoptotic, and anti-inflammatory effects, thereby protecting against loss of neuronal population and function of target cells, both *in vitro* and *in vivo* PD models[50]. Nevertheless, isolation, characterization, and testing of the biological properties of EVs retain critical issues for translation[47]. An emerging alternative to the cell-based approach is the nanotechnology approach for the targeted delivery of growth factors aiming to support and expand resident CNS stem cells for endogenous repair[51].

PROS AND CONS OF THE USE OF NSCs IN PD

Injected neural progenitor cells (NPCs) accumulate in the brain and integrate with the injured zone; however, in only a small percentage, most of the cells remained undifferentiated. The promotion of tissue survival is associated with anti-inflammatory and antiapoptotic effects at both mRNA and protein levels and increased secretion of growth factors[52] (Figure 1C). Additionally, NPCs cooperate with nearby immune and resident cells of the CNS to modulate the focal release of stem cell regulators and aid in the functional recovery from CNS injuries[53,54]. As Marsh and Blurton-Jones [55] have reviewed, there is strong evidence that NSC-mediated changes in neurotrophies resulting in increased synaptic plasticity, long-term potentiation, and neuronal survival contribute to the improvement of cognitive and motor performance observed *in vivo*. Thus, NSCs providing neurotrophic support to affected neuronal populations and synapses is a promising approach for treating neurodegenerative diseases such as PD[55]. Safety, including a potential tumorigenic effect, raised concerns. Amariglio *et al*[56] (2009) have reported a donor-derived brain tumour in a boy with ataxia telangiectasia following intracerebellar and intrathecal injection of human foetal NSCs[56]. Moreover, the complex issue of the immune rejection of transplanted cells in the brain has been demonstrated[5]. Therefore, the development of stem-cell-derived neuronal transplant therapies in PD requires consideration of this safety aspect alongside the effectiveness ones, which can be managed by addressing issues related to type and source of cells, their reproducible generation, accurate zone of transplantation and maintenance of function and viability of these grafted cells should be considered[5].

Considering transplantation safety, protocol standardisation, and the ethical aspect, approaches with the use of ESCs and IPSCs for making DAergic neurons are being developed[5,14].

ESCs, undifferentiated pluripotent cells derived from mammalian blastocysts, can make all types of cells in the body. Also, iPSCs, laboratory-generated counterparts of ESCs, that are generated from adult somatic cells, possess similar properties and can differentiate into any other cell. ESCs and iPSCs are used in many clinical trials are going on globally and have wide applications in regenerative medicine for specific diseases and conditions. The results of these trials do not, however, reach the general population because of the long and complex time frame needed to complete the clinical trials and publish the data obtained[57].

iPSCs have some advantages, such as obtaining the reprogrammed cells directly from the patients, thus potentially reducing the risk of transmissible infections and immune reactions. Additionally, they can change into any type of cell approach to treating PD[58]. Using iPSCs for treating PD is ethically permissible because patients' somatic cells can be differentiated into a pluripotent state to produce DAergic neurons implanted into the brain. Several investigations elucidated the promising role of iPSCs in regenerating DAergic neurons for treating PD. Transplantation of iPSCs ameliorated PD symptoms[58]. iPSCs have some disadvantages, such as powerful pluripotency; hence the risk of tumour development of iPSCs may be greater than other stem cells. The heterogeneity of iPSCs for clinical therapy could lead to potential tumorigenicity risks *in vivo*. Additionally, iPSCs made from autologous PD patients may contain pathogenic gene mutations that impact the outcome of cell replacement therapy[59]. Although grafted autologous iPSCs cells are considered immune-privileged, they can still be recognized as foreign (allogeneic) by the patient's immune system in some situations. Cell preparation protocol and transplantation site have been suggested to influence iPSC-derived cell immunogenicity[60]. Hence, decreasing the heterogeneity and increasing the controllability of iPSCs and cell preparation through isolation, purification, amplification, and modification of stem cells is necessary to decrease the tumorigenicity and immunogenicity potentials[61].

Although these scalable and traceable sources with well-established protocols and the efficacy in preclinical PD models have made ESCs and iPSCs promising candidates for cell-based therapy (Table 2), the risks of tumour development and immunosuppression have not been eliminated. These sources are still not ethically neutral, especially ESCs, as the embryo is used in the persevering protocol[5,14].

Despite these great efforts, there is still a gap between experimental therapeutic approaches and their translation into clinical practice. Although several clinical trials (Table 3) have been studied using cell-based treatments for PD (Clinical Trials. gov; NCT03119636[62], NCT05635409[63], NCT02452723[64], NCT03815071[65], UMIN000033564[66], NCT-03128450[67], NCT03309514[68], NCT01898390[69], NCT02795052[70], NCT00976430[71], NCT03724136[72], NCT03684122[73]) these days, the main obstacles restricting the clinical use of stem cells refer mainly to ethical concerns,

Table 2 Experimental studies on the therapeutic role of alternative stem cell sources in Parkinson's disease models

Stem cells source	Model	Effects	Ref.
hPESC line	MPTP-induced monkeys	Striatum deposition	Wang *et al*[93]
Q-CTS-hESC-1		↑ DA level	
		↑ Expression of TH-, hNCAM and GIRK2-positive cells	
		↑ Behavioral improvement	
NPCs	6-OHDA rat model	↑ DA level	Song *et al*[94]
		↑ Nurr1 and Foxa2 transcription factors	
		Improvement in behavior is not mentioned	
hiPSC lines	6-OHDA rat model	Striatum deposition	Song *et al*[95]
		↑ DAergic neurons density	
		↑ Behavioral amelioration	
MSCs	6-OHDA rat model	↑ TH-positive fibers in the striatum and TH-positive neurons in the SNpc	Wang *et al*[96]
		↑ Therapeutic effects of SDF-1α	
		↑ Behavioral amelioration	
hMSCs	6-OHDA rat model	striatum deposition	Cova *et al*[97]
		↑ DAergic neuron density	
		↑ Ki67/ PCNA – positive cells level	
		↑ Dcx expression	
		None of the grafted cells were TH- or DAT-positive	
		Improvement in behavior is not mentioned	

6-OHDA: 6-hydroxydopamine; DA: Dopamine; DAergic: Dopaminergic; DAT: Dopamine transporter; Dcx: Double cortin; GIRK2: G protein-activated inward rectifier potassium channel 2; hMSCs: Human adult MSCs; hiPSC: Human iPSC; hPESC: Human parthenogenetic ESCs; hNCAM: Human specific neural cell adhesion molecule; PCNA: Proliferating cell nuclear antigen; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NPCs: Neural progenitor cells; Ki67: Proliferating cell nuclear antigen, SDF-1α: Stromal cell-derived factor 1α; SNpc: Substantia nigra pars compacta; TH: Tyrosine hydroxylase.

immune response, tumorigenesis, and toxicity[31].

PROSPECTS FOR NSC-BASED THERAPY

From the short review above, several challenges for NSC-based therapy emerge, which can be identified at every stage of the neurosurgical procedure, including preoperative cell preparation and quality control, perioperative procedures, and postoperative graft preservation, adherence, and overall success of the therapy[74].

Strict quality control and safety assessments of the relevant cells must be in place at the preoperative stage. To achieve the optimal number of DAergic neurons, the required number of implanted cells in the final graft should be achieved [74]. The DA neurons can be derived from different stem cells or directly from NSCs. The use of foetus-derived NSCs has shown outstanding potential in rodent models of PD, and the possibility for tumorigenicity is minimal[75]. Safety issues related to the risk of tumorigenesis by grafted stem cells need to be evaluated for the long-term survival and phenotype stability of stem-cell-derived neurons in the graft following transplantation to identify the appropriate stem cell type. In addition, cell dose, case selection, and delivery route are also necessary as a precondition for the clinical use of cell-based therapies[76]. Patients receiving treatment with NSCs products are at risk for safety issues related to delivery methods, immune and allergic reactions to the medication, tumour development, heterotopic neuronal differentiation with functional disruption, and viral and microbial contamination[26]. Therefore, the fundamental concern of cell-based therapies is that before entering the clinic, transplantable cell populations must be differentiated and characterized according to good manufacturing practice regulations both *in vitro* and *in vivo*[77]. This, in turn, requires access to a large pool of differentiated cells stored in a tissue bank. However, providing sufficient *in vitro* differentiation of neurons from NSCs is challenging[78]. Cell origin is a significant problem that must be considered in treating PD by NSC transplantation as the high concentration of NSCs is needed to treat diseases[79].

Table 3 Clinical trials for cell-based therapy in Parkinson's disease

| Study identifier	Cell type	Aim of the study
NCT03119636[62]	hESC-NPCs	Safety and efficacy evaluation of intracerebral transplantation
NCT05635409[63]	hESC-DA neurons	Safety and tolerability evaluation of intraputamenal transplantation
NCT02452723[64]	ISC-hpNSC®	Safety evaluation of intracerebral transplantation
NCT03815071[65]	iPS-NSCs	Safety and efficacy evaluation
UMIN000033564[66]	iPSC-derived dopaminergic progenitors	Safety and efficacy of transplantation into the corpus striatum
NCT03128450[67]	hNSCs	Safety and efficacy evaluation of nasal delivery
NCT03309514[68]	NSC-derived neurons	Safety and efficacy evaluation of intracerebral injection
NCT01898390[69]	hVMT	Safety and efficacy evaluation
NCT02795052[70]	BMSC	Efficacy evaluation of intravenous and intranasal delivery
NCT00976430[71]	BMSC	Safety and efficacy evaluation
NCT03724136[72]	BMSC	Efficacy evaluation of intravenous and intranasal delivery on cognitive impairment
NCT03684122[73]	Umbilical cord derived MSCs differentiated into NSCs	Safety evaluation of intrathecal and intravenous injections

BMSC: Bone-marrow-derived stem cell; iPS-NSC cells: Pluripotent stem-cell-derived neural stem cells; ISC-hpNSC®: Human parthenogenetic stem-cell-derived neural stem cells developed by international stem cell corporation; hNSCs: Human neural stem cells; hVMT: Human ventral mesencephalon tissue; hESC-NPCs: Human embryonic stem cells-derived neural precursor cells.

For the promotion of the behaviour of NSCs, the fabrication of culture surfaces is developed. In this context, nanomaterials, with their nano size, unique physicochemical properties, and capability for surface functionalization, offer the potential for designed manipulation of cell behaviour. Fabricating the extracellular matrix with biocompatible nanomaterial-based scaffolds with topology simulating the microenvironment *in vivo* seems to be a promising strategy. Pandanaboina *et al*[78] demonstrated successful differentiation of embryonic rat NSCs on the nanocellulose coupled with lysine scaffold as 87% of the cells attached to the surface, and more than half of the NSCs had differentiated into functional neurons[78]. Previously they found that plasmonic gold nanorods integrated into growth surfaces tuneable stimulated and modulated embryonic rat NSCs[80]. A robust scaffold for culturing NSCs *in vitro* is three-dimensional graphene (3DG) foam, which behind supporting their growth, maintains cells in an active proliferative state through the modulation of metabolism and several metabolic processes. 3DG-stimulated pathways are involved in amino acid incorporation and glucose metabolism[81] (Figure 2).

As NSCs are regulated by a specialized vasculature during adult neurogenesis, transplanted, they may be importantly affected by the adjoining vasculature and endothelial cells[76]. Chou *et al*[82] have shown the importance of establishing an appropriate neurovascular microenvironment to deliver NSCs to replace lost DA neurons. This study indicated the molecular and cellular signalling involved in forming vasculature-like structures and increased neuronal differentiation through endothelial signalling[82]. The ability to properly locate and track transplanted NSCs is necessary to develop the efficacy of NSC therapies and ensure the treatment's safety[25].

A critical step for efficient NSCs therapy is the survival and integration of the grafted DAergic neurons, due to the lack of both stromal and growth factor support of the cells and the risk of ischaemic stress and an innate immune response [51]. Prion-like transmission synucleinopathy to the healthy neurons grafted into the brains of PD patients should be considered[18,19,21]. Nanomaterials capable of suppressing oxidative stress[83], neuroinflammation[84], or even toxic protein aggregation[85] can act as bioactive nanomedicines addressing the limitations of cell-based therapy for PD. The strategy of combining nano-enabled therapy with stem-cell-based therapy shows potential for the treatment of PD[46]. Accordingly, NSCs isolated from human olfactory bulb human coengrafted with carbon nanotubes (CNTs) restored cognitive deficits and neurodegenerative changes in the trimethyl-induced rat model of neurodegeneration. Moreover, the CNTs supported engrafted NSCs by stimulating differentiation into neurons rather than glial cells[86]. To support individual cells over the first few weeks at the postgrafting stage, attached growth-factor-loaded nanoparticles (NPs) are directly to the cell before grafting has been developed[51]. Nanomaterial carriers can be designed into "intelligent" nanodrug delivery systems functionalised with targeting ligands with the controlled release of loaded drugs/genes/cells for effective transport of them to inaccessible and specific brain areas[46]. Preclinical data have supported the safety and efficacy of the surrogate NPs stroma as no adverse effects after injection of stroma/cell constructs into the brain were evident, and human foetal ventral mesencephalic cell survival *in vivo* was increased fourfold[87]. At the postoperative stage, monitoring the injected/transplanted cells is critical for the optimisation of the neurosurgical procedure. Nanomaterials, with their unique optical properties, cellular uptake, surface functionalisation, and good biocompatibility, are
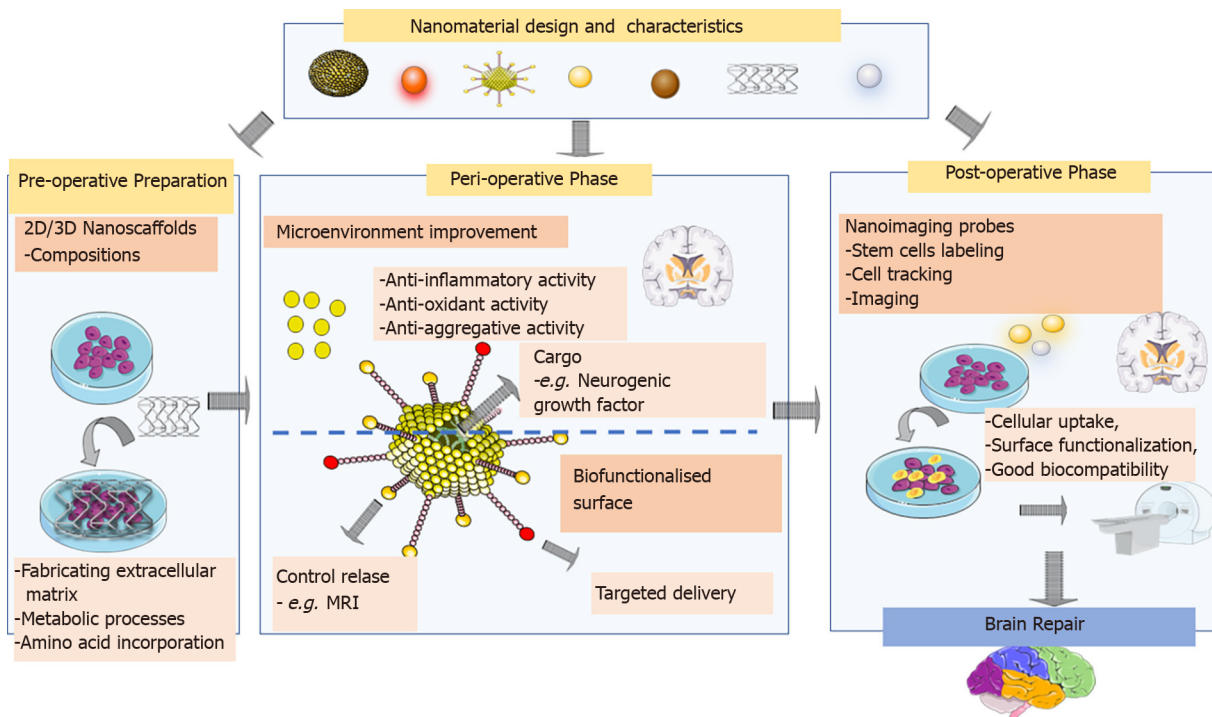
Figure 2 Application of nanomaterials in stem cell therapy of Parkinson's disease. Preoperative preparation: Nanomaterials, with their small size, unique physicochemical properties, and capability for surface functionalisation, offer the potential for designed manipulating cell behaviour. A promising strategy is that fabricating the extracellular matrix with biocompatible nanomaterial-based scaffolds with topology simulating the microenvironment. Preoperative phase: nanomaterials capable of suppressing oxidative stress, neuroinflammation, and toxic protein aggregation can act as bioactive nanomedicines addressing the limitations of cell-based therapy for Parkinson's disease. Moreover, "intelligent" nano-drug delivery systems functionalised by targeting ligands with the controlled release of loaded molecules like growth factor aims to support and expand stem cells for brain repair. At the postoperative stage, nanomaterials, with their unique optical properties, cellular uptake, surface functionalisation, and good biocompatibility, are promising candidates for cell tracking and imaging. MRI: Magnetic resonance imaging. Citation: The parts of the figures were drawn using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/Licenses/by/3.0/).

promising candidates for cell tracking and imaging[46] (Figure 2).

Advances in the development of stem cell and gene therapies create new opportunities. The combination of stem cell and gene therapy could be a technical breakthrough that increases the therapeutic effectiveness of stem cells. Engineered cells overexpressing genes involved in DA synthesis or neurotrophic factors might increase their functional capability and solve differentiation and survival issues, thus improving gene therapy's efficacy[88]. Taking into account the safety of cell-based therapy the cells can be reprogrammed to avoid as many adverse effects as possible, including immune reactions and tumours[89].

CHALLENGES FOR NANO-STEM CELL THERAPY

Nano-stem cell therapy can face many challenges, mainly related to the safety profile of NPs, including potential cytotoxicity and unknown impact on stem cell differentiation and biodegradability. There is ample scope for further research for understanding mechanisms by which cells interact with nanomaterials, the biotransformation of NPs, and their influence on cell functions, making it challenging to control the safety and effectiveness of nano-cell therapy[46,90]. Since the activity of NPs strongly depends on their sizes, structures, shapes, and surface chemistry and is cell type dependent[91], several problems related to methods for the synthesis, bio-functionalisation, characterisation, and tailoring of 3D nanostructures in tissue engineering should be addressed[46,90].

Given the challenges and state-of-art accomplishments of nanotechnology, it can be expected to lead to the development of nano-stem cell therapy for PD. It is supported by recently merged multifunctional nanotheragnostic approaches for effective stem cell therapy in managing brain cancers[92].

CONCLUSION

PD significantly affects society because it carries a mounting socioeconomic burden, while no cure is currently available. The studies discussed in this review have shown the potential of NSC-based therapy for PD (Figure 1C). Although NSCs present major promise in treating this neurodegenerative disease, they raise significant concerns. They may form a

tumour, which is a more devastating condition than PD. Moreover, immunological rejection, ethical issues, limited cell sources, and complications related to skill to maturation into desired neuronal cell types and proper differentiation are important reasons for further research to facilitate NSC therapy in PD.

Currently, combining stem cell therapy, including genetically engineered cells, with nanotechnology approaches to complement each other's advantages provides new insights into the improving the therapy protocol and efficacy. The safety profile and the efficacy of nano-stem cell therapy in treating neurodegenerative diseases is a research hotspot. It is believed that future advancements in new nanomaterials will surely benefit NSCs-based therapy from this technology.

In conclusion, although various obstacles and challenges remain, nano-stem cell therapy holds promise to cure PD. Clearly, its continuous improvement and development from the stage of laboratory experiments to the clinical application are necessary.

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FOOTNOTES

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