NEUROSCIENCE

REVIEW

X. Guo et al. / Neuroscience 463 (2021) 370–382

# Current Developments in Cell Replacement Therapy for Parkinson's Disease

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**Abstract—Parkinson's disease (PD) is characterized by tremor, rigidity, and bradykinesia. PD is caused mainly by depletion of the nigrostriatal pathway. Conventional medications such as levodopa are highly effective in the early stage of PD; however, these medications fail to prevent the underlying neurodegeneration. Cell replacement therapy (CRT) is a strategy to achieve long-term motor improvements by preventing or slowing disease progression. Replacement therapy can also increase the number of surviving dopaminergic neurons, an outcome confirmed by positron emission tomography and immunostaining. Several promising cell sources offer authentic and functional dopaminergic replacement neurons. These cell sources include fetal ventral mesencephalic tissue, embryonic stem cells (ESCs), neural stem cells (NSCs), mesenchymal stem cells (MSCs) from various tissues, induced pluripotent stem cells (iPSCs), and induced neural cells. To fully develop the potential of CRT, we need to recognize the advantages and limitations of these cell sources. For example, although fetal ventral midbrain is efficacious in some patients, its ethical issues and the existence of graft-induced dyskinesias (GID) have prevented its use in large-scale clinical applications. ESCs have reliable isolation protocols and the potential to differentiate into dopaminergic progenitors. iPSCs and induced neural cells are suitable for autologous grafting. Here we review milestone improvements and emerging sources for cell-based PD therapy to serve as a framework for clinicians and a key reference to develop replacement therapy for other neurological disorders.** 

**Key words: Parkinson's disease, cell replacement therapy, fetal ventral mesencephalic tissue, TRANSEURO, stem cells, cellular reprogramming.**

## INTRODUCTION

In the 19th century, physicians characterized Parkinson's disease (PD) by a range of noteworthy motor symptoms, such as rest tremor, muscle rigidity, and bradykinesia. With increasing understanding of PD, we have uncovered related nonmotor features, such as olfactory dysfunction, cognitive deficit, and depression (Kalia and Lang, 2015).

Medications such as levodopa, dopamine agonists, monoamine oxidase inhibitors, and other neuroprotective agents are highly effective in the early stage of PD. For instance, selegiline and rasagiline, always used in combination with α-tocopherol, are beneficial for PD patients at an early stage (Finberg and Rabey, 2016).

Dopamine agonists, such as rotigotine and bromocriptine, can relieve bradykinesia and rigidity. Dopamine replacement therapy, such as administration of levodopa, enhances intracerebral dopamine concentrations and reliably mitigates tremor, bradykinesia and rigidity, and anticholinergic drugs diminish tremor. However, the mainstays of treatment, represented by dopaminergic drugs, have the significant limitation that they fail to stop the underlying neurodegeneration. Moreover, the effectiveness of dopaminergic drugs diminishes with development of the disease, followed by L-dopa-induced dyskinesia and other motor fluctuations in advanced stages.

Additionally, surgical treatments, such as stereotactic ablations and deep brain stimulation, have been developed to partially overcome the shortage of medication and manage the severe motor complications in advanced PD patients. However, neither surgical approach becomes the root and branch procedure (Fasano et al., 2012). Accordingly, strategies have been investigated that are aimed at rebuilding the pathway and reshaping the brain. For example, dopaminergic neurons or the pluripotent cells have been placed in the striatum.

In the early phase of preclinical work, investigators sought to develop a cell-based therapy with fetal ventral mesencephalon (fVM). Animal studies conducted from 1977 to 1985 yielded considerable encouraging results in the 6-OHDA or MPTP lesion model that employed

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https://doi.org/10.1016/j.neuroscience.2021.03.022
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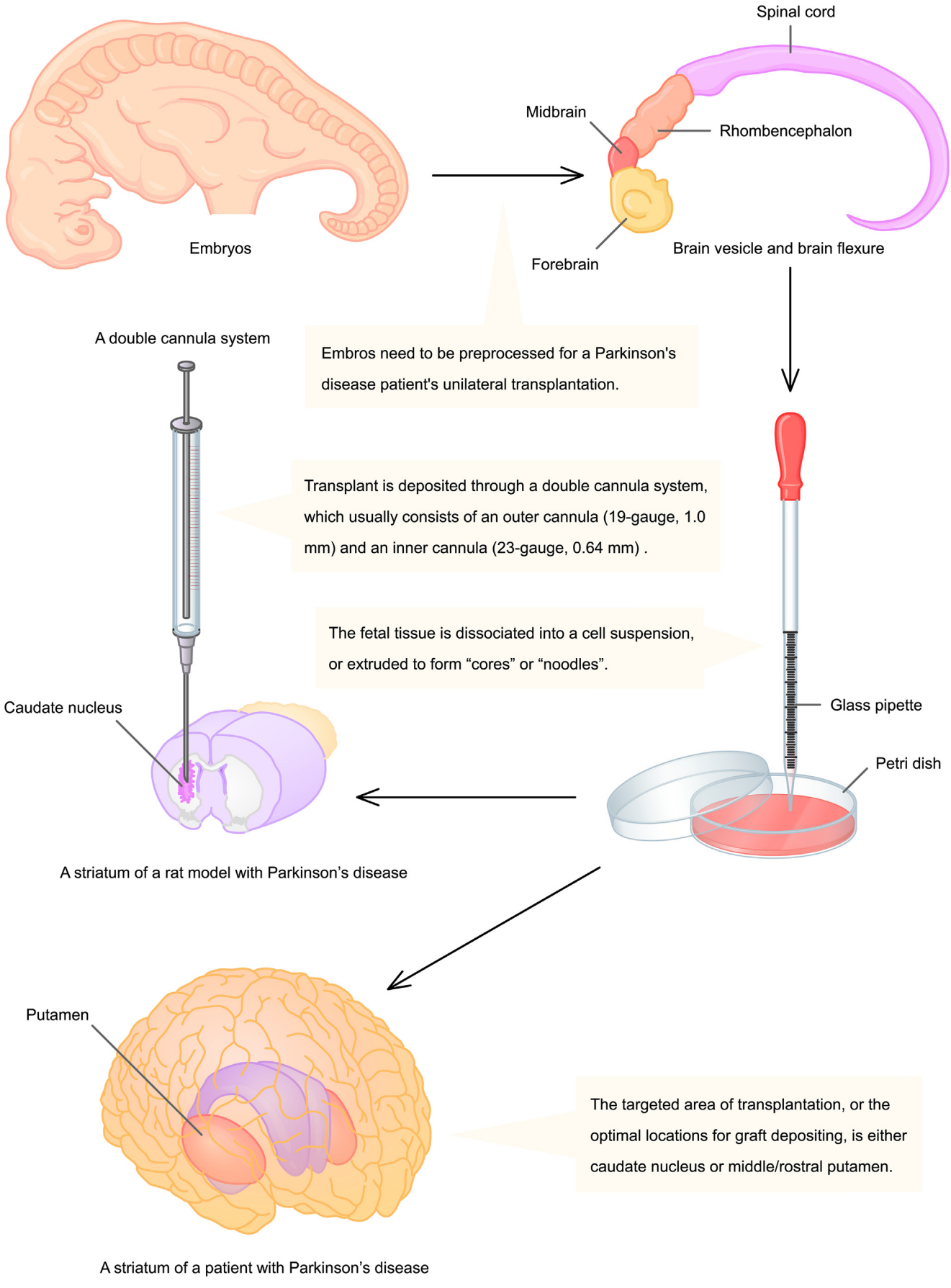

Spinal cord
Midbrain
Rhombencephalon
Forebrain
Embryos
Brain vesicle and brain flexure
Embros need to be preprocessed for a Parkinson's disease patient's unilateral transplantation.
A double cannula system
Transplant is deposited through a double cannula system, which usually consists of an outer cannula (19-gauge, 1.0 mm) and an inner cannula (23-gauge, 0.64 mm) .
The fetal tissue is dissociated into a cell suspension, or extruded to form “cores” or “noodles”.
Glass pipette
Petri dish
Caudate nucleus
A striatum of a rat model with Parkinson’s disease
Putamen
The targeted area of transplantation, or the optimal locations for graft depositing, is either caudate nucleus or middle/rostral putamen.
A striatum of a patient with Parkinson’s disease

either human or mouse fetal tissue. The transplanted cells survived in the lesion area, which demonstrated the incredible plasticity of brain (Parmar et al., 2019). On the basis of these observations, since 1987, investigators have conducted a series of clinical trials, the first of which was transplantation performed in Sweden – a country with a liberal policy towards embryonic research (Kupsch and Oertel, 1994; Lindvall et al., 1988; Parmar et al., 2019). From 1987 to 2003, a diverse array of open label trials and double-blind placebo-controlled trials showed that, in general, fVM transplantation partially worked and relieved tremor to some extent in most patients. However, for a minority of patients, the method failed to show a measurable benefit or, worse, it caused unexpected complications (Parmar et al., 2019).

Consequently, these highly variable outcomes have prompted a large-scale multicenter trial titled TRANSEURO (NCT01898390). The University of Cambridge and the University of Lund designed the trial after a period of downturn in fetal tissue transplantation (Kirkeby et al., 2017). The aim of the project is to create more convincing and feasible protocols for fetal tissue transplantation, including a series of detailed steps of receptor selection, graft preparation, tissue implantation, and post-grafting medicine. The trial will end in 2021 (Barker et al., 2019; Kirkeby et al., 2017).

Meta-analysis has shown reductions in complications, such as on and off phenomenon, after fVM transplantation. Human fetal ventral midbrain-based therapy is considered efficacious because some patients improve motor function after grafting. However, this therapy was banned for a long time because most of the graft was obtained from donation by women who underwent abortion.

For the safer applications and a standardized protocol, as well as medical ethics, investigators have turned to other cell sources. Breakthroughs made with stem cell have encouraged investigators to use embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) as the scalable and traceable source. The well-established protocols for ESCs and the efficacy in preclinical PD models have made ESCs an excellent candidate for cell-based therapy. The risks of tumor development and immunosuppression have not been eliminated. Without doubt, iPSCs bring the risk of tumor formation. However, induced neurons directly reprogrammed from somatic cells possess lower capacity for tumorigenicity.

## FVM TRANSPLANTATION

With a time-frame of 30 years, experiences with tissue preparation, the route and target of transplantation, immunotherapy, and patient selection from fVM studies have greatly contributed to the development of cell-based PD therapy. Moreover, the standardized procedure of fVM trials that yielded excellent graft outcomes has been transferred as an indicator for new trials that have tested other grafting cell types.

Between 1987 and 1995, diurnal motor fluctuation, such as the ‘‘on and off” phenomenon, has been mitigated or relieved to varying degrees after surgery for about 150 patients. Although L-dopa treatment has not been withdrawn root and branch, the effect or reaction of which manifestly lasts longer with reduction of drug-induced side effects (Lindvall, 1995).

For instance, Lindvall et al. recruited four severely advanced PD patients for an open-label trial between 1987 and 1989. However, two of the patients merely had limited amelioration of syndrome (Lindvall et al., 1989). Accordingly, the procedure/protocol has been improved (with a thinner cannula from 2.5 mm to 1.0 mm, medium transferred from saline to HBSS, more advanced loading system) and then served for the other two patients for whom motor function improved significantly and symptomatic relief was sustained (Lindvall et al., 1990, 1992).

More specifically, a 49-year-old patient, one of the four cases, who had severe rigidity and tremor in the right arm for 11 years, received unilateral transplantation with fetal tissue from four embryos (8–9 weeks gestational age). The tissue was dissociated into a cell suspension by digestion with trypsin and implanted into the left putamen along the anteroposterior axis at three sites separated by 2 mm. The preoperative drug schedule included 700 mg L-dopa, 10 mg bromocriptine, and 6 mg benzhexol chloride; this schedule was maintained during the first five months after transplantation.

Before surgery, clinicians rated the patient as stage III, which indicated a middle stage of PD on the scale of Hoehn and Yahr. By the second month after surgery, the patient’s rigidity and dyskinesia were ameliorated. Positron emission tomography with [$^{18}$F]-dopa showed increased [$^{18}$F]-dopa uptake in the left caudate nucleus and putamen (Lindvall et al., 1990).

More interestingly, one of the four Lindvall patients whose motor impairment was the most alleviated (not the 49-year-old) has been followed for 10 years. The benefits of this transplantation lasted at least 10 years; the patient had a high quality of life with only mild Parkinson’s syndrome even though the patient withdrew from L-dopa treatment after 32 months. Furthermore, in accordance with the clinical outcome, positron emission tomography with [$^{18}$F]-dopa indicated that the [$^{18}$F]-dopa uptake nearly reached the normal level three years after transplantation. The [$^{11}$C]-RAC binding potential with/without methamphetamine inducing procedure also reached a normal level (Piccini et al., 1999).

It seems that the efficacy of implantation depends mainly on the source of transplantation graft, the route

**Fig. 1.** Schematic of fetal tissue transplantation for brain repair in Parkinson’s disease. Usually included in the trials are patients with idiopathic or drug-induced Parkinson’s disease who are responsive to levodopa. Fetal midbrain tissue is collected from the brain vesicle of a 4-week embryo. The fetal tissue is dissociated into a cell suspension, or extruded to form ‘‘cores” or ‘‘noodles”. Then the tissue is placed with a stereotactic system into the targeted area, which is either caudate nucleus or middle/rostral putamen.

of implantation, and the number of fetuses (Fig. 1) (Kirkeby et al., 2017). For instance, Freed et al. analyzed the data from earlier studies and concluded that 7–8-week embryos were the best for grafting, which they used for their randomized controlled trial (Freed et al., 2001). In addition, the quality of the fetal tissue needs to be ensured by screening for herpes and cytomegalovirus. Moreover, the donor will also be excluded if she has been infected or contaminated by any form of hepatitis B and C, syphilis, and human immunodeficiency virus (Breeze et al., 1995). In addition, the general health of the gravida must be supervised because it may affect results.

The fetal dopaminergic-rich tissue is usually dissociated into a cell suspension (Lindvall et al., 1990) or extruded to form" cores" or" noodles" (Freed et al., 2001). If choosing ‘‘cores", the thickness of the ‘‘core" should be optimum for the grafted neurons to extend the neurotic outgrowth covering the putamen, neither too thick to survive the inner layer of cells nor too thin to form synapses with host neurons. Accordingly, evidence indicates that a diameter of 0.33 mm will promote neuronal survival in the graft (Breeze et al., 1995). In addition, bilateral implantation may lead to a better clinical outcome compared with unilateral implantation. Choosing five target sites within the neostriatal caudate-putamen per side can enhance the innervation around each target site (Fig. 2) (Barker et al., 2019; Hagell et al., 1999). The tissue needs to be fresh, which means it cannot be stored longer than four days at 4 °C (Parmar et al., 2019). It must be maintained in tissue culture medium (Hibernate-E with lazaroids, or F12 medium with human placental serum, or HBSS) (Barker et al., 2019; Kelly et al., 2011).

Less than 20% of cells within grafts survive and extend axons around transplant deposits. This survival limit is caused mainly by apoptosis associated with caspase 3 and calpain-activated necrosis. Calpain-activated necrosis usually occurs from 90 min to 6 weeks after transplantation, whereas caspase 3 apoptosis occurs during 1–2 weeks (Emgard et al., 2003).

In addition, JNK (c-Jun N-terminal kinases) inhibitors and caspase inhibitors such as lazaroids have exhibited neuroprotective properties, and, by inhibiting some apoptosis pathways, they increased survival of dopaminergic neurons during tissue preparation, tissue implantation, and interaction with the host neurons. Glial cell line-derived neurotrophic factor (GDNF) alone or in combination with basic fibroblast growth factor and insulin-like growth factor-I has been documented to improve survival of transplanted cells (Zawada et al., 1998).

The condition of receptors may influence the outcome of transplants. It is better to recruit patients aged < 60 (Freed et al., 2001). Kirkeby et al. defined an ideal patient for fVM transplantation as one who meets the following requirements: younger than 65 years, with a PD duration less than 10 years, without any L-dopa induced dyskinesia, without psychosis, and without any significant diminution of dopaminergic neurons other than substantia nigra (Kirkeby et al., 2017).

The factors that influence survival of grafts may also include storage condition and reagents for immunosuppression, such as cyclosporine and azathioprine (Barker et al., 2013).

There are about five million dopaminergic neurons in the substantia nigra of a healthy person (Kopyov et al., 1997). Alleviation of PD syndrome is based on an aggregate of 100,000 dopaminergic neurons per side, and with a minimal number of serotonin neurons. Serotonin (5-hydroxytryptamine) is a biogenic amine most noted for its function as a neurotransmitter (Emgard et al., 2003). Björklund and Lindvall, 2000) reported a need of 80,000 functional dopaminergic neurons for a positive outcome.

Diverse approaches have been developed to assess the efficacy of transplantation, including positron emission tomography, modified Hoehn and Yahr scale, unified PD rating scale, dosage of postoperative medication, and postmortem analysis (Evans et al., 2012). Postmortem analysis offers direct proof of the survival and growth of transplanted cells. In Freed's research, two patients were subjected to postmortem analysis. One patient showed that about 63,000 dopaminergic neurons survived and formed 2–3 mm long synapses in the 7th month after transplantation. Another patient had 43,000 surviving dopaminergic neurons in the 3rd year after transplantation (Freed et al., 2001).

Another issue that needs to be eliminated is postoperative complications such as hemorrhage and graft-induced dyskinesias (GID). GID always occurs one year after transplantation. GID is caused mainly by overgrowth of the dopaminergic neurons and inappropriate location or uneven density of graft deposits (Carlsson et al., 2006). A high dopaminergic to serotonin ratio may also cause GID.

## STEM CELL-BASED THERAPIES FOR PD

Compared with fVM, a diverse array of stem cells is apparently more prospective and more likely to be produced in large scale. Encouraged by changing and advancing technologies of cell derivation and cell differentiation, more investigators have tested stem cells in PD clinical trials (Barker et al., 2017). With sufficient convincing data from preclinical trials, international specialists are currently considering how to standardize the operation and the cost effectiveness, instead of feasibility.

Stem cells have advantages such as self-renewal and plasticity in forming an array of tissues. Accordingly, they are acceptable replacements for damaged nonrenewable neurons, especially to treat PD. Classically, stem cells are characterized as either ESCs or adult stem cells according to different stages of differentiation. Stem cells can also be categorized according to the degree of developmental potency, i.e., totipotent, pluripotent, and unipotent (De Los Angeles et al., 2015). Stem cells used to treat PD are usually classified as ESCs, neural stem cells (NSCs), mesenchymal stem cells (MSCs), and iPSCs.

Recently, in place of fetal tissue, human embryonic stem cells (hESCs) have been used successfully for grafts. These cells are derived from multipotent stem cells, or embryoblasts, that are the inner cell mass from one side of the blastocyst. Human ESCs are pluripotent

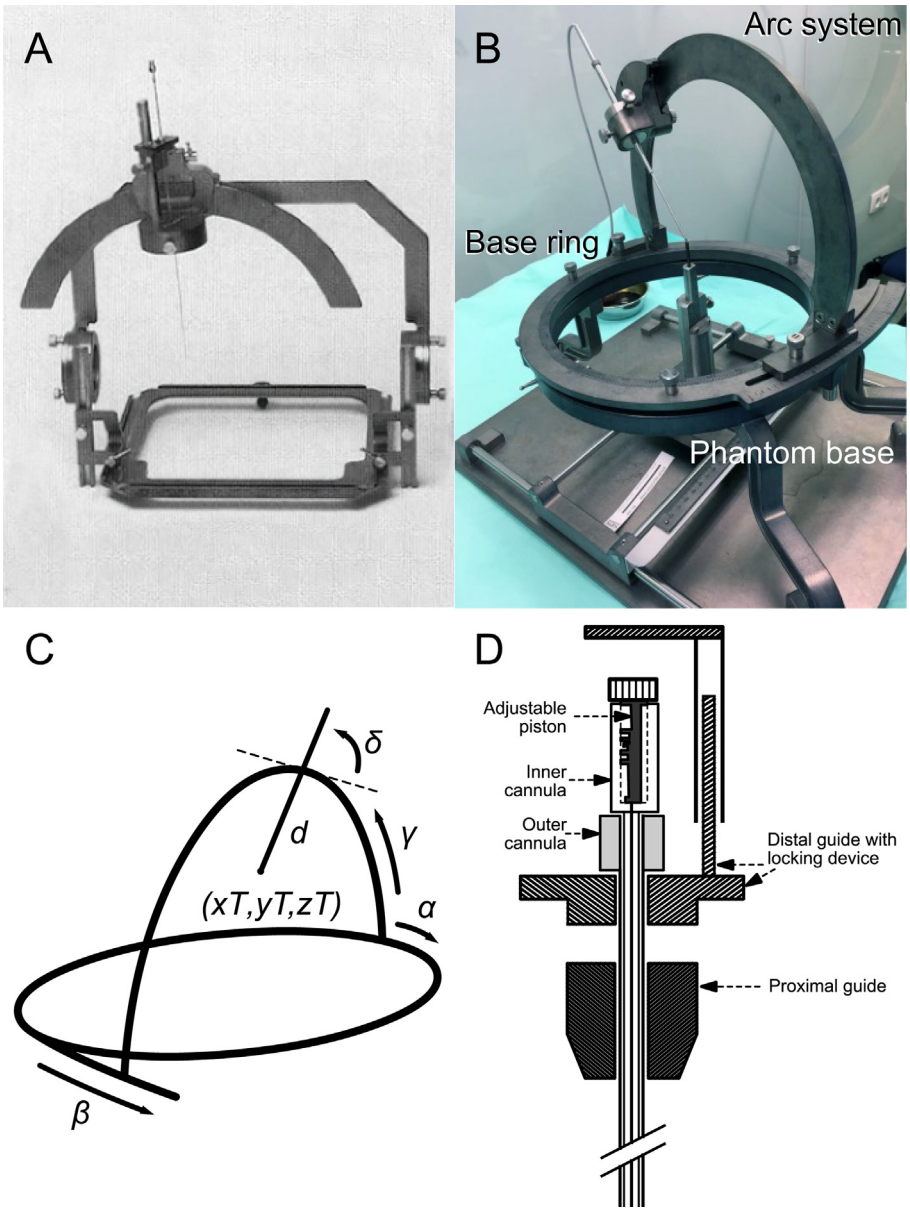



**Fig. 2.** A technical note for the stereotactic apparatus and double-cannula system used in CRT. The Brown-Roberts-Wells (BRW) stereotactic system was used in the first fVM transplantation led by Freed (Breeze et al., 1995). In addition to BRW system, the Leksell system and CRW system are also used widely for cell-based therapy. The BRW frame can be separated into four main components: (1) a base ring affixed to the skull; (2) an N-localizer device to assist CT scanning; (3) an arc system to assist the surgery; (4) a phantom base for maintenance and calibration (Khedr et al., 2018). In the BRW system, a cerebral target point is depicted by five parameters (α, β, γ, δ, d), which contain four angles α, β, γ, δ, and the insertion depth d (Khedr et al., 2018). Transplant is deposited through a double cannula system, which usually consists of an outer cannula (19-gauge, 1.0 mm) and an inner cannula (23-gauge, 0.64 mm) (Lindvall et al., 1989). Permission to reuse and Copyright. In Fig. 2, graphic a is adapted with permission from Freed et al. (Breeze et al., 1995) ©1995 Congress of Neurological Surgeons, graphics b and c are adapted with permission from Khedr et al. (Khedr et al., 2018) ©2018 Cureus Inc, and graphic d is adapted with permission from Lindvall et al. (Lindvall et al., 1989) ©1989 American Medical Association.

stem cells in the primitive undifferentiated stage with two key properties: self-renewal and multilineage differentiation (Pera et al., 2000). These properties are due to telomerase that elongates telomeres and retards aging of hESCs (Hiyama and Hiyama, 2007).

In addition, human embryonic germ cells (hEGCs), another pluripotent stem cell derived from embryonic gonad, are mentioned in the literature together with hESCs because they have properties similar to hESCs (Pera et al., 2000). Similarly, hESCs and hEGCs have comparable morphology, similar functions, similar markers/antigens, and similar growth requirements (Pera et al., 2000). For example, hESCs and hEGCs are characterized by a set of cell surface biomarkers, namely, alkaline phosphatase, stage-specific embryonic antigen-3 and -4, and both cell types express multiple transcription factors, such as octamer-binding protein OCT-4, that maintain pluripotency (Nagano et al., 2008; Pera et al., 2000). However, intriguingly, stage-specific embryonic antigen-1 is expressed in hEGCs derived from genital ridges, but it is not expressed in hESCs; thus, stage-specific embryonic antigen-1 can identify pluripotent stem cells derived from primordial germ cells (Nagano et al., 2008).

Since the first of ESC lines were established in 1998, they have been investigated widely and used in diverse fields of medicine and pharmacology, such as production of neurons to cure neurodegenerative diseases, production of islet β cells to cure type-1 diabetes mellitus, and solid organ transplantation (Thomson et al., 1998). A standard embryonic stem/germ cell line must be derived from a multipotent cell population, such as an embryoblast, it must maintain a normal karyotype *in vitro*, it can be propagated/expanded serially and indefinitely *in vitro*, and it can differentiate into an array of tissues and somatic cells. However, there is a difference between murine ESCs and human ESCs that relates to developmental potency. Murine ESCs can differentiate into tissues of all three germ layers, whereas the development potency of human ESCs is restricted (Pera et al., 2000). In addition, because ESCs have a high tumorigenic potential in the undifferentiated state, they cannot be implanted directly into substantia nigra. A well accepted approach is to differentiate ESCs

**Table 1.** Preclinical studies of cell-based therapy with UC-MSCs

| Study | PD model (experimental grouping) | Differentiation Protocol | Transplantation Method | Outcome (endpoint) |
|---|---|---|---|---|
| Fu et al. (2006) | Thirty-six SD rats were lesioned by 6-OHDA and divided equally into three groups | Three-step method using NCM + 500 ng/ml SHH + 100 ng/ml FGF8 | Unilateral stereotactic injection into the striatum; about $1 \times 10^5$ cells per rat, without any immunosuppression therapy | Graft cells derived from hUC-MSs by NCM + SHH + FGF8 decreased the number of amphetamines induced-rotations, whereas the graft cells cultured with only NCM failed to produce any significant change |
| Weiss et al. (2006) | Female SD rats received toxic 6-OHDA and were divided into two groups: hUC-MSC transplant group and sham transplant group. | Without any dopaminergic neuronal differentiation | Unilateral stereotactic injection into the striatum, without any immunosuppression therapy | hUC-MSCs survived and caused 50% reduction of apomorphine-induced rotations in some of the transplanted PD rats within 12 weeks. One special result was that the increase in TH positive cells (DA neurons) was found not only in the transplanted side, but also found in the contralateral side in some transplanted rats. |
| Xiong et al. (2010) | Eighteen rats in the hUC-MSC transplant group; twelve rats in the sham surgery/saline group | Without any dopaminergic neuronal differentiation | Stereotactic injection. About $1 \times 10^6$ cells per rat. hUC-MSCs labeled with DiI, without any immunosuppression therapy | Some transplanted hUC-MSCs migrated from CPu to SNc, VTA and contralateral cerebral hemisphere. There was a 67.48% reduction of apomorphine-induced rotations in the transplant group; no epileptogenic effect was found in this study |
| Xiong et al. (2011) | Rotenone-induced PD rats divided into three groups | 0.5 mM RA + 20 ng/ml EGF + 50 ng/ml bFGF + 50 ng/ml NGF | Cells were infected with Ad-VEGF-EGFP before transplantation. Infection by Ad-EGFP used as a control. Stereotactic injection; $1 \times 10^6$ cells per rat | VEGF protein expressed in the graft cells enhanced their therapeutic effects based on comparison between Ad-VEGF-EGFP group and Ad-EGFP group |
| Mathieu et al. (2012) | 6-OHDA-lesioned male Wistar rats | Without dopaminergic neuronal differentiation | A stereotactic injection into the left substantia nigra; $2 \times 10^4$ cells per rat | DA neurons ($TH^+$ cells) increased by 20% in the hUC-MSCs transplant group compared with PD rats with sham surgery. hUC-MSCs might not cause host neuroprotection. An alleviation of syndrome was shown in both cylinder test and adjusting step test |
| Kang et al. (2013) | 6-OHDA-lesioned C57BL/6 mice | In vitro neurogenic differentiation with 10 ng/ml bFGF, 200 μM butylated hydroxyanisole, 2 mM valproic acid, 10 μM forskolin, 5 μg/ml insulin, 1 μM hydrocortisone, 25 mM KCl | An injection into the left substantia nigra with stereotaxic system with about $2 \times 10^4$ porcine UC-MSCs per mouse | NGF, nestin, VEGF, IL-6 were expressed by porcine UC-MSC transplants that promote therapeutic effects. Bridge test showed motor improvement in transplanted mice, whereas the rotarod test failed to show improvement |
| Shetty et al. (2013) | Male 6-OHDA-lesioned SD rats | Differentiate in vitro with a cocktail of growth factors/supplements including 2 ng/ml bFGF, 100 ng/ml NGF, 50 ng/ml Noggin, 200 μM BHA | An injection into substantia nigra with stereotactic system (28-gauge syringe) with about $1 \times 10^6$ cells per rat | Among the three cell sources of undifferentiated UC-MSCs, undifferentiated BM-MSCs, and differentiated UC-MSCs, differentiated UC-MSCs significantly reduced the number of apomorphine-induced rotations and contained the largest TH + population. In addition, efficacy lasted up to 48 weeks |

(*continued on next page*)

**Table 1** (*continued*)

| Study | PD model (experimental grouping) | Differentiation Protocol | Transplantation Method | Outcome (endpoint) |
|---|---|---|---|---|
| Aliaghaei et al. (2016) | Test group: four rats first receive toxic 6-OHDA and then differentiated UC-MSCs as a treatment | Pre-differentiation step: 0.5 μM RA + 5 ng/ml FGF2; Differentiation step: incubate with conditioned medium from CPECs | Stereotactic injection; about $1 \times 10^5$ cells per rat | **1.** CPECs expressed a cocktail of growth factors, including GDNF, BDNF, NGF, FGF2, and VEGF (RT-PCR analysis). **2**. Some UC-MSCs were differentiated to DA neurons by RA, FGF2, and conditioned medium from CPECs (RT-PCR analysis). **3.** The number of rotations was reduced in the test group within four weeks |
| Wang et al., 2016 | Female unilateral PD rat model induced by 6-OHDA | Just a precondition of exposure to neurotrophic factors for three days. Results proved that hUC-MSCs can differentiate *in vivo* | Stereotaxic injection with hUC-MSC suspensions; six target sites and $6 \times 10^5$ cells per rat | **1.** Immunohistochemistry: more TH positive cells found in hUC-MSC group within eight weeks. **2.** Rotational behavior diminished after CRT. **3.** DA contents two months after transplantation:1754.12 ± 14.79 pg/g (hUC-MSC group) **versus** 1687.94 ± 14.82 pg/g (control group) |
| Zhao et al. (2016) | Male SD rats induced by 6-OHDA | 50 μg/ml L-ascorbic acid, 250 ng/ml SHH, 100 ng/ml FGF8, 50 ng/ml bFGF, 50 ng/ml BDNF | Injection into right caudate putamen with stereotaxic system. About $1 \times 10^6$ cells for five target sites per rat | **For hUC-MSCs group**, more Hsp60 expression and less rotational behavior. **For DA group,** more Hsp60 expression and less rotational behavior |
| Jinfeng et al. (2016) | The study was designed from a cellular and molecular perspective. All the PC12 cells lesioned by MPTP and used as a PD cell model were divided into four groups | The concentrated CUR-activated hUC-MSC supernatant promoted differentiation of PC12 cells into DA neurons | No transplantation; used conditioned medium (cell supernatant of hUC-MSCs activated by CUR) to culture the lesioned PC12 cells | The detection results in PC12 cell model after hUC-MSC-CUR treatment showed significant effect of antioxidation |

Abbreviations: NCM = neuron-conditioned medium. SHH = sonic hedgehog. BDNF = brain-derived neurotrophic factors. FGF8 = fibroblast growth factor 8. bFGF = basic fibroblast growth factor. NGF = nerve growth factor. BHA = butylated hydroxytoluene. MSC = mesenchymal stem cell. hUC-MSCs = human umbilical cord-derived mesenchymal stem cells. BM-MSCs = bone marrow-derived mesenchymal stem cells. 6-OHDA = 6-hydroxydopamine. MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. CRT = cell replacement therapy. SD = Sprague - Dawley. Hsp60 = heat shock protein 60. CUR = curcumin. MAP2 = microtubule associated protein 2. NO = nitric oxide. iNOS = induced nitric oxide synthase. PC12 cell line was established from the pheochromocytoma of Rattus norvegicus. RA = retinoic acid. CPECs = choroid plexus epithelial cells. FGF2 = fibroblast growth factor 2. GDNF = glial cell line-derived neurotrophic factor. VEGF = vascular endothelial growth factor. EGF = epidermal growth factor. DiI = 1,1′-dioctadecyl-3,3,3′3′-tetramethylindocarbocyanine perchlorate. CPu = corpus striatum. SNc = substantia nigra pars compacta. VTA = ventral tegmental area. EGP = enhanced green fluorescent protein. Ad-VEGF-EGFP = replication-deficient adenovirus vector carrying both VEGF gene and EGFP gene. Ad-EGFP = replication-deficient adenovirus vector containing EGFP gene. Lmx1α = the LIM homeobox transcription factors 1 alpha. NTN = Neurturin; Msx1 = Msh homeobox 1.

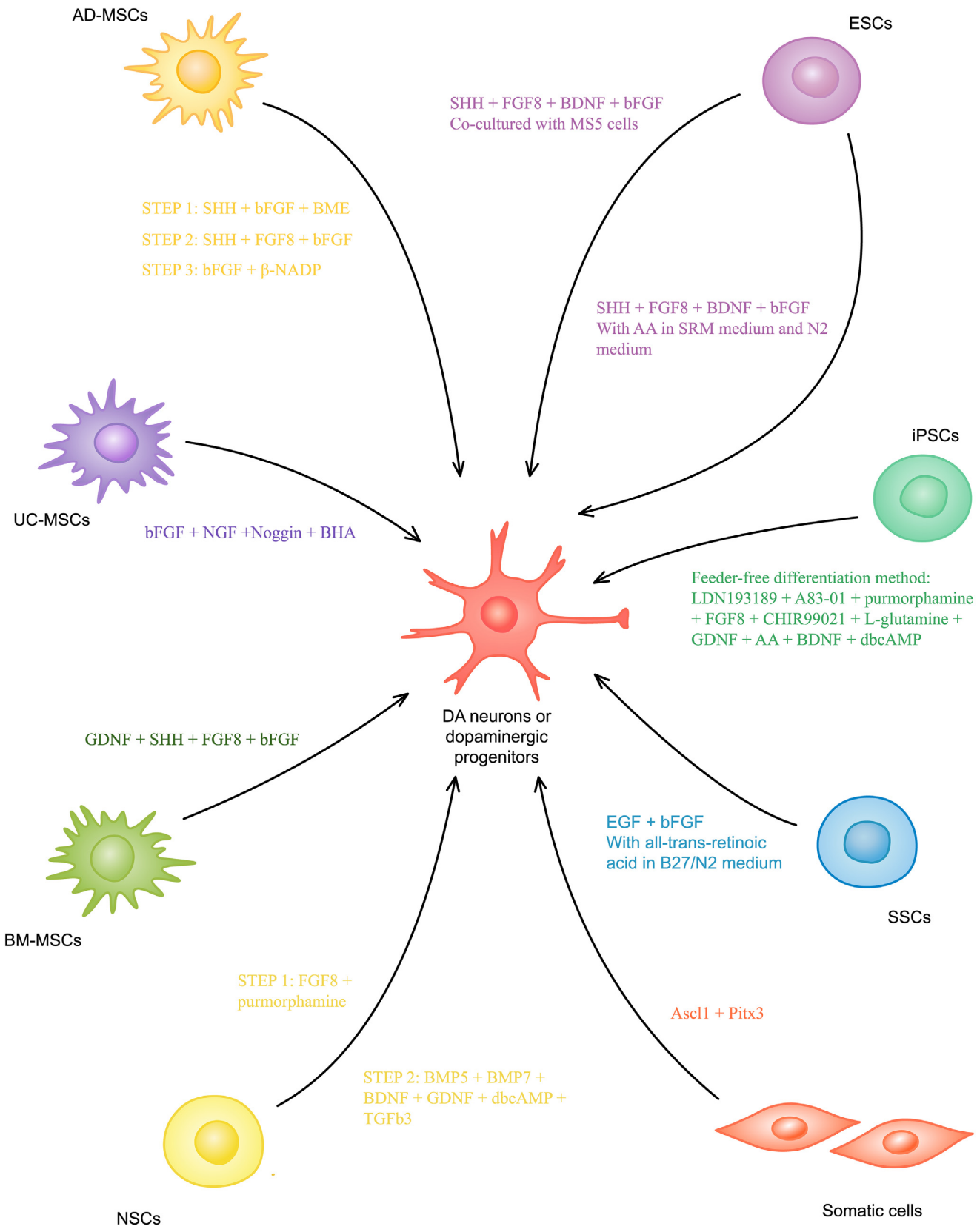
into dopaminergic neurons with a combination of fibroblast growth factor-8, sonic hedgehog, and brain-derived neurotrophic factor. As well, ESCs must be co-cultured with MS5 cells (Kriks et al., 2011; Perrier et al., 2004).

Human ESCs have been investigated extensively. However, not until 2007 were hESCs induced into dopaminergic neurons, transplanted in a PD model, and demonstrated to have a predicted outcome, confirming the feasibility of using hESCs for PD research (Rodríguez-Gómez et al., 2007).

NSCs can differentiate into specific neurons, neuroglial cells, and oligodendrocytes. In addition, NSCs are competent for direct implantation into the degenerating region. In general, NSCs are found in forebrain subventricular zone, sub granular zone of dentate gyrus, medulla spinalis, and striatum, with a dormant state in vivo (Gage, 2000). Therefore, NSCs are isolated usually from the subventricular zone or striatum of adult humans or mice. NSCs also can be isolated from fetal tissue or derived from human umbilical cord blood (Jablonska et al., 2010).

Under the microscope, MSCs share a morphology with fibroblasts. In general, MSCs can differentiate into cells/tissues that are supposed to be derived from a different embryonic germ layer. So far, MSCs can be isolated and expanded at large scale from bone marrow, umbilical cord blood, amniotic fluid, and adipose tissue.

Bone marrow mesenchymal stem cells (BM-MSCs) can be used for autogenic transplantation, hence to reduce immune exclusion. Furthermore, BM-MSCs have


AD-MSCs
ESCs
SHH + FGF8 + BDNF + bFGF
Co-cultured with MS5 cells
STEP 1: SHH + bFGF + BME
STEP 2: SHH + FGF8 + bFGF
STEP 3: bFGF + β-NADP
SHH + FGF8 + BDNF + bFGF
With AA in SRM medium and N2 medium
iPSCs
UC-MSCs
bFGF + NGF +Noggin + BHA
Feeder-free differentiation method:
LDN193189 + A83-01 + purmorphamine + FGF8 + CHIR99021 + L-glutamine + GDNF + AA + BDNF + dbcAMP
DA neurons or dopaminergic progenitors
GDNF + SHH + FGF8 + bFGF
EGF + bFGF
With all-trans-retinoic acid in B27/N2 medium
SSCs
BM-MSCs
STEP 1: FGF8 + purmorphamine
Ascl1 + Pitx3
STEP 2: BMP5 + BMP7 + BDNF + GDNF + dbcAMP + TGFb3
NSCs
Somatic cells

been used in gene therapy. Zhang et al. inserted the GDNF gene into the genome of BM-MSCs and implanted the modified cells into the striatum and substantia nigra of PD monkeys. The autologous BM-MSCs survived, grew well in vivo, and alleviated syndrome (Ren et al., 2013).

Venkataramana et al. enrolled seven PD patients in their open-label trial for stereotactic unilateral transplantation with autogenic BM-MSCs. They not only confirmed the safety of the novel method, but also they enabled two patients to significantly reduce the dose of L-dopa (Venkataramana et al., 2010).

Compared with BM-MSCs, umbilical cord blood-derived MSCs (UC-MSCs) are collected more easily without an invasive procedure, and maintained in a more primitive state (Van Pham et al., 2016). Cell surface markers/antigens of human UC-MSCs are many, such as SH2, SH3, CD29, CD73, CD44, CD166, CD105, and CD90. Furthermore, SH2 and SH3 are efficient and convenient for identification of UC-MSCs (Boroujeni and Gardaneh, 2017). Under different culture conditions, many morphologies can form, for example, fusiform cells, polygonal cells, and pyramidal cells. Among all types of MSCs, UC-MSCs have the highest anti-inflammatory effect via Ang-1 (Jin et al., 2013). Following an increasing array of preclinical studies that confirm the safety and efficacy of UC-MSC transplantation, at least one clinical trial is ongoing in China (as identified by ClinicalTrials.gov, the Registry number is NCT03550183).

One thing we would like to point out here is, the detailed information of researches summarized in table formats for ESCs (Shroff et al., 2017), NSCs (Ganz et al., 2011), BM-MSCs (Gugliandolo et al., 2017), and iPSCs (Amin et al., 2019) has already been provided in other reviews on this topic. For this reason, we only advocate a table rarely seen in other published reviews summarizing the important UC-MSCs transplantation studies in recent 15 years, which include 11 preclinical studies (Table 1). In this table, we list the detailed protocols of UC-MSC differentiation and the main results of each study so that the discrepancy among these studies can be easily compared.

Adipose tissue MSCs have also been popular in biomedical research. However, current isolation strategies have difficulties in delivering a pure population of adipose tissue MSCs that meet the needs of brain research, which is the main obstacle to their use in clinic.

Induction of dopaminergic neurons has been important in application and development of cell replacement therapy (CRT) (Fig. 3). A classic method is to culture stem cells with a combination of FGF8, SHH, and wingless-type MMTV integration site family member 1 (Wnt1). Both FGF8 and Wnt1 participate in inducing neuronal differentiation in the distal region of the ectoderm along the anterior-posterior axis, which includes the natural differentiation of dopaminergic neurons. Furthermore, SHH is expressed in cerebral ventricular zone and FGF8 is produced by the isthmus, which is the border between midbrain (mesencephalon) and hindbrain (deuterencephalon). Both SHH and FGF8 function in various dopaminergic neuron-inducing methods (Smidt and Burbach, 2007). For example, SHH and FGF8, in cooperation with bFGF, can effectively promote the induction of dopaminergic neurons from human amniotic fluid MSCs (Phonchai et al., 2019).

A range of neurotrophic factors promotes survival and growth of induced dopaminergic neurons, such as nerve growth factor, GDNF, and BDNF. Culture medium supplemented with GDNF, bFGF, SHH, and FGF8 can help MSCs differentiate into differentiating neurons (Trzaska and Rameshwar, 2011); Moreover, GDNF combined with leukemia inhibitory factor can induce dopaminergic neurons from neuronal stem cells (De Los Angeles et al., 2015).

## CELLULAR REPROGRAMMING FOR PD

We are aware of the enormous potential of stem cell-based therapy by a train of preclinical trials, which have involved considerable effort. However, we fear the formation of teratomas caused by a small portion of impaired cells. A relentless march of cellular reprogramming technology offers a solution to this problem.

The concept of cellular reprogramming differs from that of gene recombination. Cellular reprogramming is a revolutionary technology that uses a combination of diverse transcription factors to modify genes when they are expressed. Gene reprogramming does not involve altering the coding sequence. Although gene recombination is a more mature technology, both cellular reprogramming and gene recombination are important genetic engineering methods.

The particular combination of transcription factors determines whether a gene is activated or silenced. The transcription factors regulate all aspects of transcription, for example, when to express, how to express, and for how long to express.

Takahashi and Yamanaka (2006) reported the world-shaking result that iPSCs could be produced by (only) four transcription factors, Oct3/4, Sox2, Klf4, and c-Myc. Fur-

**Fig. 3.** A schematic of different stem cell differentiation into dopaminergic neurons. The text in the Figure refers to the defined signaling factors, transcription factors, and other conditions to induce dopaminergic neurons from different pluripotent cell sources and popular sources of cell-based therapy. Abbreviations: AD-MSCs = adipose tissue-derived mesenchymal stem cells. BM-MSCs = bone marrow-derived mesenchymal stem cells. UC-MSCs = umbilical cord-derived mesenchymal stem cells. SSCs = spermatogonial stem cells. bFGF = basic fibroblast growth factor. NGF = nerve growth factor. BME = β-Mercaptoethanol. FGF8 = fibroblast growth factor 8. β-NADP = β-nicotinamide adenine dinucleotide phosphate hydrate. SHH = sonic hedgehog. BDNF = brain-derived neurotrophic factors. AA = ascorbic acid. N2 = culture medium specifically for neurons, without serum. SRM = serum replacement medium. B27 = serum-free neurobasal medium. BMP5 = bone morphogenetic protein 5. BMP7 = bone morphogenetic protein 7. TGFb3 = transforming growth factor beta 3.

thermore, in 2007, these investigators delivered exactly same transcription factors by retroviruses to reprogram human fibroblasts into iPSCs (Takahashi et al., 2007). The four transcription factors have since been used widely as a classical method. And new methods have been examined to ensure effectiveness and safety for future clinical applications (Hanna et al., 2010).

Some new combinations of transcription factors, for example, Sall4, Nanog, Esrrb, and Lin28, have effectively induced cells. In addition, some groups are using new vectors instead of retroviruses to generate iPSCs (Buganim et al., 2014).

Cellular reprogramming also ushers in a new era for the treatment of neurological disorders, especially PD. Since the inception of iPSCs, neurologists have been increasingly interested in producing the ideal graft material to substitute for impaired neurons. For example, Kikuchi et al. (2017) grafted human iPSC-derived dopaminergic progenitors into the striatum of PD model monkeys. These grafted dopaminergic neurons, which contained tyrosine hydroxylase, successfully survived and covered the entire putamen and part of the caudate head of three monkeys in the experimental group. The monkeys also showed improvements in PD scores. In addition, Kikuchi et al. indicated that dopaminergic neurons derived from patients and healthy individuals survived well and function in a similar way. In another study, Morizane et al. (2017) reported that, in major histocompatibility complex-matched monkeys, the neurons derived from primate iPSCs survived for a long time with less immune response. The first clinical trial with iPSCs-derived neurons for PD is in progress at the Center for iPS Cell Research and Application of Kyoto University (Takahashi, 2017).

Lastly, scientists are performing studies of direct reprogramming, a new technology to reprogram fibroblasts directly into specific neurons by different combinations of driving or inducing factors and omitting the primitive undifferentiated stage. This new procedure tends to lower the tumor initiating capacities because it skips the intermediate pluripotent state.

In 2016, about 1.4 million people had PD in China. From 1990 to 2016, China had the largest increase in the age-adjusted prevalence of PD, which mirrored rapid industrialization (Dorsey et al., 2018). However, existing clinical therapies have been limited to temporarily alleviating the parkinsonian syndrome, that is, the therapies do not prevent the underlying pathological process.

Fortunately, CRT, which have been a hotspot for the therapeutic research of PD, not only help alleviate symptoms, but also they help to slow the disease course, that is, with a rapidly growing knowledge of CRT, we are more likely to reduce the economic burden of PD and fill the gap left by medication therapy.

This review provides a glimpse into the thirty-year progression and main sources of CRT. We discuss the conclusion of existing methods and clinical trials that used fetal tissue, the exploration of a series of stem cells as graft candidates for efficient transplantation, and the emerging capacity of cellular reprogramming for both efficiently generating iPSCs and deriving dopaminergic neurons from somatic cells.

The principal sources of CRT are fetal ventral mesencephalon (hfVM), ESCs, NSCs, MSCs, iPSCs, and induced functional dopaminergic neurons (iDAs).

The first potent graft source for CRT was hfVM. Except for the widely known debate on ethical issues, extensive studies of fetal tissue implantation, especially TRANSEURO, have led to a better understanding of the optimized transplantation process, and studies have placed PD CRT on the verge of clinical application. On the basis of robust clinical data from hfVM trials, investigators have derived the details of all types of protocols, and technological issues and considerations on how to achieve better outcomes have been discussed in depth in this review.

ESCs are another potential cell source to reverse neurodegeneration and motor impairment. Prior studies demonstrated that existing modified protocols of differentiation not only achieve a high midbrain DA neuron yield, but also they avoid neural overgrowth and tumorigenic cells derived from inappropriate differentiation of ESCs. What is more, two ongoing large clinical trials have used ESCs, namely, EUROPEAN STEM-PD and NYSTEM-PD.

NSCs also work as a therapeutic tool because they spontaneously migrate into the degenerating area and drive endogenous neurogenesis.

Besides ESCs and NSCs, MSCs are also a type of derived pluripotent stem cells that are easily obtained from multiple sources with high ethical acceptance. On the basis of different origins, investigators divide MSCs into bone marrow-derived MSCs (BM-MSCs), umbilical cord blood-derived MSCs (UC-MSCs), amniotic fluid-derived MSCs (AF-MSCs), and adipose tissue-derived MSCs (AT-MSCs). The transplanted MSCs exert therapeutic effects in two ways. They differentiate toward dopaminergic neurons and then survive and function in the substantia nigra or following intrastriatal transplantation. Alternatively, MSCs release factors by paracrine activity and alter the cellular environment. Basically, all MSCs exhibit low teratoma risk, low immunogenicity, and a potential for autologous transplantation.

More specifically, BM-MSCs have been the most explored. They are isolated by invasive techniques and harvested in limited amounts. UC-MSCs are more primitive and exhibit higher proliferative capacity compared with BM-MSCs. AT-MSCs are more easily accessible and have a higher yield compared with BM-MSCs.

Cell reprogramming has arisen from the need to overcome the shortcomings of MSCs and other stem cells as the graft source for cell-based therapy. The shortcomings include mainly uncertainty about proliferation and differentiation, especially for adult stem cells.

In addition to the evolving cell reprogramming technologies, a variety of technical factors associated with CRT also affect a diverse array of neurological

cases, especially for PD. Soon, the combination with the most advanced equipment, for example, the robotized stereotactic assistant system and the ultrasonic based neuro-navigation system, will definitely help to meet the challenge of mitigating or reversing ongoing degeneration.

Apart from the equipment, injectable biomaterial scaffolds are advantageous to the survival and proliferation of dopaminergic neurons within grafts. Encapsulation of a neural cell suspension in an injectable hydrogel loaded with neurotrophic factors can reduce both apoptotic cell death and recruitment of host immune cells (Moriarty et al., 2019).

CRT is a major advance in the management of PD, and it is reaching clinical trials. Nevertheless, cell-based therapies have limitations. For example, there is usually a lack of evidence to show amelioration of nonmotor symptoms, that is, CRT fails to gradually stop extrastriatal disease progression.

Beside CRT, deep brain stimulation, DA synthetic gene therapies, and growth factor gene therapies are frequently compared and discussed for the treatment of PD. As compared to these three therapies, CRT is seemed like the most promising therapy for its ability of establishing the most consistent DA delivery, as well as the successful cases of integrating the grafted cells with host circuitry. Although it will take a long time for developing related technologies and yield enough clinical data, CRT is the hope for mid-to-late PD patients.

To date, we have produced unlimited amounts of functional dopaminergic neurons or progenitors from a series of safe and accessible cell sources. At the same time, data from long-term clinical trials are being collected and assembled by G-Force PD and Chinese academic groups. G-Force PD is a consortium that involves teams from Europe, USA, and Japan. It has already met twice to discuss common problems, solutions, and the roadmap to the clinic (Barker et al., 2015). Hopefully, CRT will be initiated into extensive clinical application by a combination of novel cell sources and more advanced biomolecular technologies, and CRT will continue to contribute to PD therapy.

In the long perspective, personalized medicine and patient-specific cell sources will increasingly enable CRT as the core therapy of PD. Standardized protocols for cell isolation, examination and in vitro expansion facilitate the process of obtaining market approval. By that time, the needs of PD patients will be best met after considering all of the contributing factors such as patients' conditions, the disease progress and patients' compatibilities with autologous or allogeneic transplantation.

## CONFLICT OF INTEREST

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## AUTHOR CONTRIBUTIONS

X.T. contributed to conception, design, supervision, and revision of the study. X.G. wrote the first draft of the manuscript. L.T. revised the manuscript for intellectual content.

## FUNDING

This work was supported by grants from National Natural Science Foundation of China (Grant No. 81271298).

## ACKNOWLEDGMENTS

The authors thank AiMi Academic Services (www.aimieditor.com) for English language editing and review services. The authors also thank Peng Du for his dedication to the figures in the manuscript.

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*(Received 26 November 2020, Accepted 17 March 2021)*
*(Available online 26 March 2021)*