

Combined cell-based therapy strategies for the treatment of Parkinson's disease: focus on mesenchymal stromal cells

Jannette Rodríguez-Pallares^{1,2,*}, María García-Garrote^{1,2}, Juan A. Parga^{1,2}, José Luis Labandeira-García^{1,2,*}<https://doi.org/10.4103/1673-5374.350193>

Date of submission: April 12, 2022

Date of decision: May 16, 2022

Date of acceptance: June 2, 2022

Date of web publication: August 2, 2022

From the Contents

Introduction	478
Search Strategy and Selection Criteria	478
Historical Overview	478
Co-Grafting Strategies: a Review	479
The Potential of Mesenchymal Stromal Cells for Cell Therapy	480
Mesenchymal Stromal Cells and Neural Repair	480
Mesenchymal Stromal Cell-Based Therapies for Parkinson's Disease	481
Conclusions and Future Perspectives	481

Abstract

Parkinson's disease is a neurodegenerative condition characterized by motor impairments caused by the selective loss of dopaminergic neurons in the substantia nigra. Levodopa is an effective and well-tolerated dopamine replacement agent. However, levodopa provides only symptomatic improvements, without affecting the underlying pathology, and is associated with side effects after long-term use. Cell-based replacement is a promising strategy that offers the possibility to replace lost neurons in Parkinson's disease treatment. Clinical studies of transplantation of human fetal ventral mesencephalic tissue have provided evidence that the grafted dopaminergic neurons can reinnervate the striatum, release dopamine, integrate into the host neural circuits, and improve motor functions. One of the limiting factors for cell therapy in Parkinson's disease is the low survival rate of grafted dopaminergic cells. Different factors could cause cell death of dopaminergic neurons after grafting such as mechanical trauma, growth factor deprivation, hypoxia, and neuroinflammation. Neurotrophic factors play an essential role in the survival of grafted cells. However, direct, timely, and controllable delivery of neurotrophic factors into the brain faces important limitations. Different types of cells secrete neurotrophic factors constitutively and co-transplantation of these cells with dopaminergic neurons represents a feasible strategy to increase neuronal survival. In this review, we provide a general overview of the pioneering studies on cell transplantation developed in patients and animal models of Parkinson's disease, with a focus on neurotrophic factor-secreting cells, with a particular interest in mesenchymal stromal cells; that co-implanted with dopaminergic neurons would serve as a strategy to increase cell survival and improve graft outcomes.

Key Words: brain repair; cell replacement; co-grafts; dopaminergic neurons; fetal ventral mesencephalic tissue; mesenchymal stem cells; neural grafting; neural transplantation; neuroblasts; neurotrophic factors

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the presence of motor manifestations such as tremor, rigidity, bradykinesia, and postural instability and a variety of non-motor symptoms that include sensory abnormalities, autonomic dysfunction, sleep disturbances, fatigue, apathy, and psychiatric disorders (Armstrong and Okun, 2020). PD pathology mainly lies in the degeneration of dopaminergic neurons in the substantia nigra (Poewe et al., 2017). Although there is a wide range of pharmacological treatments for PD, all treatments currently available provide only symptomatic relief. Levodopa is the most potent and effective treatment and remains as the gold standard. However, the beneficial effect of levodopa gets progressively shorter throughout the disease, and prolonged treatment is associated with drug-induced dyskinesia and other disabling side effects that limit its use (Armstrong and Okun, 2020; Lopez-Lopez et al., 2021). With a relatively localized neural degeneration, PD is considered a good target for cell therapy (Fan et al., 2020). Over nearly four decades, different restorative surgical therapies have emerged to replace lost dopaminergic neurons. First clinical trials based on intrastriatal transplantation of human fetal ventral mesencephalic (fVM) tissue showed that grafted cells survive and induce significant, long-lasting improvements in motor symptoms (Lindvall et al., 1990), although possible effects on non-motor symptoms were not explored. However, the low availability of human mesencephalic tissue limited its use. Currently, stem cells represent a promising alternative source for the generation of dopaminergic neurons (Jang et al., 2020; Rahimpour et al., 2022). In addition to important issues such as defining an optimal cell source, better criteria for PD patient selection, improved and standardized protocols, avoiding rejection, and promoting functional integration (Rahimpour et al., 2022), other factors that compromise the survival of grafted dopaminergic neurons, such as mechanical trauma, growth factor deprivation, hypoxia, oxidative stress, and neuroinflammation need to be addressed (Brundin et

al., 2000; Moriarty et al., 2017). Low dopaminergic survival can be partially counteracted by neurotrophic factor addition. Although different strategies have been studied, direct, timely, and controllable delivery of neurotrophic factors into the brain faces important challenges (Gantner et al., 2020; Studer and Tabar, 2020). Different types of cells secrete neurotrophic factors constitutively and co-transplantation of these cells with dopaminergic neurons represents a feasible strategy to increase neuronal survival. In this review, we focus on the co-grafting of dopaminergic neurons and neurotrophic factor-secreting cells, with a particular interest in mesenchymal stromal cells (MSCs), as a possible strategy to reduce cell death after grafting, which represents one of the drawbacks faced by cell therapy to make the definitive leap to the clinic (Figure 1).

Search Strategy and Selection Criteria

The search strategy and selection criteria were limited to articles published in peer-reviewed journals. A literature review of articles published in English from inception up to April 2022 was conducted by searching in the National Library of Medicine (PubMed) database (<https://pubmed.ncbi.nlm.nih.gov/>) using the following keywords: fetal ventral mesencephalic tissue, graft, Parkinson's disease, co-grafts, mesenchymal stromal/stem cells, transplantation, neuroblasts, survival, embryonic stem cells, pluripotent induced stem cells and combination of the above terms. We also revised references of eligible articles.

Historical Overview

In 1975, Seiger and Olson grafted fetal brain tissue into the anterior eye chamber, laying the groundwork for future transplants in the nervous system. Cells derived from fVM tissue, enriched in dopaminergic cells, were the first type of cells transplanted in an animal model of PD. These initial preclinical

¹Laboratory of Cellular and Molecular Neurobiology of Parkinson's Disease, Research Center for Molecular Medicine and Chronic Diseases (CiMUS), IDIS, Department of Morphological Sciences, University of Santiago de Compostela, Santiago de Compostela, Spain; ²Networking Research Center on Neurodegenerative Diseases (CIBERNED), Madrid, Spain

*Correspondence to: José Luis Labandeira-García, MD, PhD, joseluis.labandeira@usc.es; Jannette Rodríguez-Pallares, PhD, jannette.rodriguez@usc.es.

<https://orcid.org/0000-0001-6865-2433> (Jannette Rodríguez-Pallares); <https://orcid.org/0000-0001-7155-7234> (María García-Garrote);

<https://orcid.org/0000-0003-2072-5642> (Juan A. Parga); <https://orcid.org/0000-0002-8243-9791> (José Luis Labandeira-García)

Funding: This work was supported by grants from Consellería de Cultura, Educación e Ordenación Universitaria, Xunta de Galicia (ED431G/05, ED431C 2018/10), European Regional Development Fund (FEDER), Instituto de Salud Carlos III (RD16/011/0016; RD21/0017/0031), Secretaría de Estado de Investigación, Desarrollo e Innovación (Grant/Award, number RTI2018-098830-B-I00) (to JLLG).

How to cite this article: Rodríguez-Pallares J, García-Garrote M, Parga JA, Labandeira-García JL (2023) Combined cell-based therapy strategies for the treatment of Parkinson's disease: focus on mesenchymal stromal cells. *Neural Regen Res* 18(3):478-484.

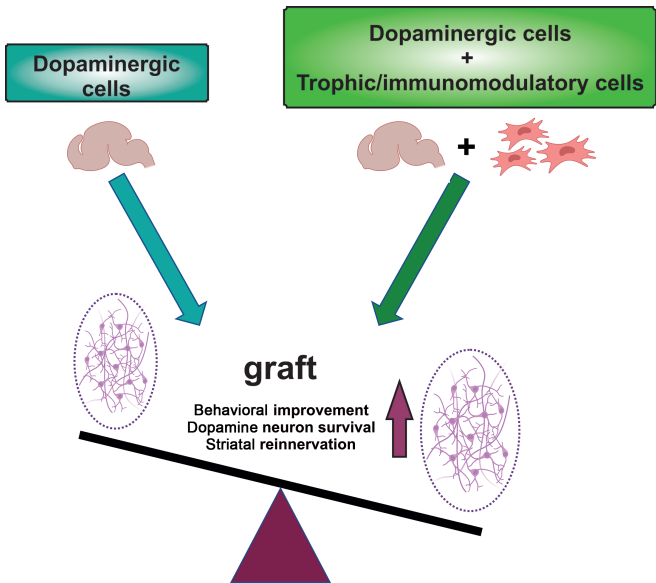


Figure 1 | Co-grafting of dopaminergic cells with trophic/immunomodulatory cells as a Parkinson's disease cell therapy.

Co-transplantation strategies are proposed as an approach to improve behavioral deficits, dopamine neuronal survival, and striatal reinnervation. Created with BioRender (<https://biorender.com/>).

studies confirmed that dopaminergic grafts were able to survive, extend projections into the host striatum and ameliorate behavioral deficits in dopamine-depleted animals (Bjorklund et al., 1980). Such was the impact of these results that in the early 1980, the first PD patients in the world received intracerebral grafts. However, these patients were grafted with autologous chromaffin cells obtained from the adrenal medulla, which had also been used in animal models of PD, to avoid the practical and ethical issues associated with human fetal tissue (Freed et al., 1981). Although it was initially assumed that adrenal medulla and fVM cells had similar mechanisms of action based on dopamine production, further studies showed that adrenal medulla graft benefits were due to neurotrophic actions that promote the sprouting of striatal dopaminergic fibers and not to dopamine production (Freed et al., 1990). However, the transient and highly variable improvements observed both in animal models and in PD patients, the frequent surgical complications, and the low cell survival observed in post-mortem studies led to the abandonment of the use of adrenal medullary tissue for transplantation (Date et al., 1990). A breakthrough in the field was the use of dissociated cell suspensions derived from fVM tissue, which permitted the targeting of deep structures as well as multiple graft placements (Bjorklund et al., 1983). A crucial step forward in clinical translation was the demonstration that dopaminergic neurons isolated from human embryonic/fetal tissue survive, provide extensive graft-derived striatal innervation, and ameliorate motor deficits after transplantation in preclinical models of PD (Brundin et al., 1986). Based on almost 10 years of transplantation studies in animal models, the first patients receiving grafts of human fVM tissue were implanted in 1987 (Lindvall et al., 1990). This pioneering study demonstrated for the first time that fVM grafted cells can survive in a human brain affected by neurodegenerative disease and could restore dopamine levels, monitored in the striatum using [¹¹C]-raclopride positron emission tomography, for as long as 10 years after surgery (Piccini et al., 1999). Interestingly, post-mortem studies carried out 24 years after grafting showed dense graft-derived reinnervation in the putamen (Hallett et al., 2014). The outcomes of a series of open-label trials showed promising results and generated high expectations in the field, leading the way to two National Institutes of Health-funded, double-blind, randomized, placebo-controlled trials. However, the results were disappointing, with some grafted patients showing normalized dopamine signaling while other participants showed modestly or no recovery compared to patients subjected to sham surgery (Freed et al., 2001). Nevertheless, even more discouraging was the fact that a subset of grafted patients developed graft-induced dyskinesias years later (Hagell et al., 2002), possibly due to the composition of the transplanted cell suspension. Experimental studies using different proportions of dopaminergic and serotonergic neurons in the cell suspension showed that higher proportions of serotonergic neurons, which are usually present in the graft, led to the dyskinesias, since serotonergic terminals could release dopamine as a false transmitter in an unregulated fashion (Politis et al., 2010). Additional evidence suggests that endogenous serotonergic neurons and serotonergic and dopaminergic interactions would contribute to the development of graft-induced dyskinesias (Muñoz et al., 2020). With the knowledge acquired in subsequent research, it is now considered that these studies were carried out prematurely and some of the side effects could have been avoided through careful selection of patients, improving the method of implantation and the composition of the grafted cell suspension, and optimizing the post-transplant immunosuppressive regimes (Bjorklund and Lindvall, 2017). The cumulative reanalysis of previous trials led a European research consortium called TRANSEURO to design a step-by-step optimized

open-label trial, which is currently ongoing, with the main objective of developing an efficacious and safe methodology for fVM cell-based therapy for PD (ClinicalTrials.gov identifier NCT01898390) (Barker and TRANSEURO consortium, 2019).

Due to the abovementioned shortage of human fVM tissue for cell-based therapy and advances in stem cell knowledge, many efforts have focused on generating dopaminergic neurons for transplantation from stem cells, which potentially offer an unlimited number of cells. Different stem cell types have been considered as a possible source of neurons for the treatment of PD. However, the best positioned seem to be embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) (Jang et al., 2020). Notable advances in differentiation protocols have led to the achievement of highly pure dopaminergic neurons suitable for clinical trials that showed satisfactory therapeutic effectiveness in animal models of PD and did not cause tumors in these animals (Xiong et al., 2021). The first transplantations using clinically graded hiPSC-derived neurons have already been carried out (Schweitzer et al., 2020). Piao et al. (2021) have recently reported the generation of the first ESC-derived dopaminergic cell product for the treatment of PD, which was approved for a clinical trial in the United States (Takahashi, 2021; Figure 2). There is no doubt that stem cell technologies have the potential to be at the forefront of such PD treatments (Parmar et al., 2020). The results of ongoing and upcoming trials would be highly anticipated since the success of these trials would open up new possibilities for using cell therapy for the treatment of PD (Fan et al., 2020). However, it should be noted that, beyond the motor symptoms, the disease exhibits non-motor symptoms that can be also disabling for the patient, which currently are not targeted by cell therapy and should also be considered in future therapies (Pantcheva et al., 2015).

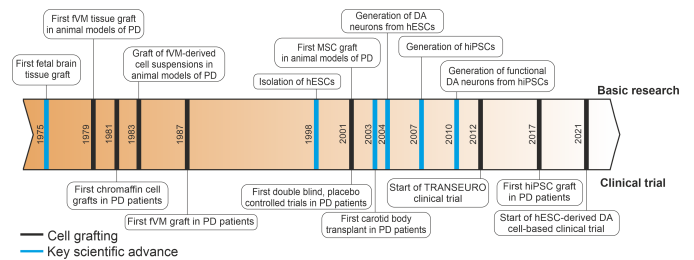


Figure 2 | Timeline of breakthroughs in cell therapy for Parkinson's disease.

Relevant contributions in the field of cell therapy are shown in black. Key scientific advances are shown in blue. DA: Dopamine; fVM: fetal ventral mesencephalic; hESCs: human embryonic stem cells; hiPSCs: human induced pluripotent stem cells; MSC: mesenchymal stromal cell; PD: Parkinson's disease.

Despite the encouraging results obtained, cell-based therapies face important limitations and challenges. In addition to issues concerning safety and reproducibility, a critical aspect is the survival rate of the grafted dopaminergic neurons. Different evidence suggests that only 3–20% of grafted dopaminergic cells survive after the procedure (Brundin et al., 2000; Kim et al., 2020; Li and Li, 2021; Hiller et al., 2022). Triggers that may initiate dopaminergic cell loss in grafts include mechanical trauma, neuroinflammation, poor vascularization, and growth factor deprivation in the host brain (Barker et al., 1996; Brundin et al., 2000; Moriarty et al., 2017). The critical interval during which most dopaminergic cells die is the first few days after transplantation (Sortwell et al., 2000). The idea of utilizing cells with neurotrophic properties to modify the pathologic brain environment or promote neuroprotective effects has been appearing on the horizon for central nervous system disorders. In the following section, we will review the most relevant studies in which combinations of dopaminergic grafts with other cells with supportive properties have been used as a strategy to overcome the poor survival of transplanted neurons (Figure 3).

Co-Grafting Strategies: a Review

Co-grafting strategies based on the combination of fVM tissue with cells with neurotrophic properties that could promote the survival of transplanted dopaminergic cells represent a therapeutic option to overcome the typical loss of 80–95% of the grafted dopaminergic neurons following transplantation. In the 1980s, the first studies exploring the benefits of peripheral nerve transplantation on the survival of dopaminergic grafts were carried out. Schwann cells, the principal glial cell type in the peripheral nervous system, are involved in neuronal survival and nerve regeneration (Ma et al., 2021). Aguayo et al. (1984) demonstrated that transplanted fVM neurons in 6-hydroxydopamine (6-OHDA)-lesioned rats survived, and extended axons guided by sciatic nerve grafts. Different groups also demonstrated that co-grafts of fVM tissue with peripheral nerves or Schwann cells induced behavioral improvements and promoted axonal growth to the striatum compared to fVM grafts in rat models of PD (van Horne et al., 1991; Timmer et al., 2004). However, other studies did not observe neuroprotective effects after co-transplantation of fVM tissue and saphenous nerve in lesioned monkeys (Collier et al., 1994). Olfactory ensheathing cells (OECs) are another type of peripheral glial cells specialized in supporting continuous olfactory nerve fiber growth throughout adulthood. Co-culture of OECs with nigral cells promoted dopaminergic neurite outgrowth (Denis-Donini and Estenez, 1988). Implantation of cultured OECs in dopamine-depleted rats after 6-OHDA

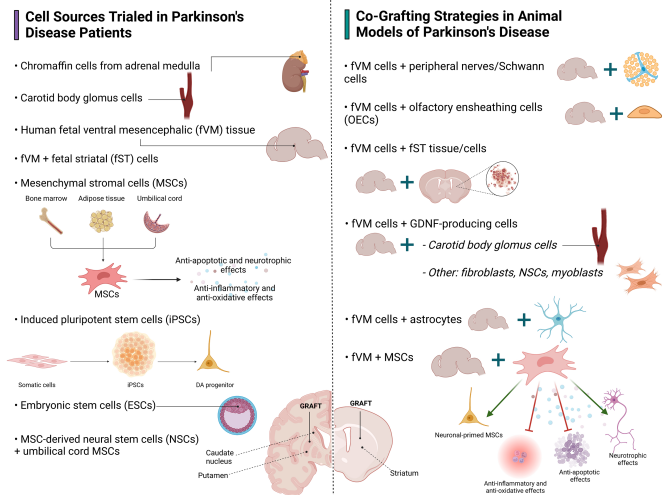


Figure 3 | Cell-based therapies tried in patients and co-grafting strategies tested in animal models of PD.

The left-hand-side of the figure shows cell sources used in clinical trials in PD patients and the right-hand-side shows co-grafting strategies tested in animal models of PD. ESCs: Embryonic stem cells; fST: fetal striatal; fVM: fetal ventral mesencephalic; GDNF: glial cell line-derived neurotrophic factor; iPSCs: induced pluripotent stem cells; MSCs: mesenchymal stromal cells; NSCs: neural stem cells; OECs: olfactory ensheathing cells; PD: Parkinson's disease. Created with BioRender (<https://biorender.com/>).

lesioning was not sufficient to promote tissue repair or functional recovery (Dewar et al., 2007). However, the combination of fVM with OECs enhanced the efficacy of grafted neurons in different rat models of PD (Agrawal et al., 2004; Johansson et al., 2005; Weng et al., 2017). Similar results were obtained when dopaminergic neurons isolated from porcine fVM cells or dopaminergic neurons derived from neural stem cells were co-implanted with OECs (Shukla et al., 2009; Weng et al., 2020).

It is well-established that striatal cells exert both tropic and trophic effects that contribute to providing a target for mesencephalic dopaminergic neurons during the development of the nigrostriatal pathway (Barker et al., 2020). To extend these properties to cell transplantation, several works focused on studying the effects of co-grafting striatal cell suspensions with fVM tissue. In a ground-breaking study carried out by Brundin and collaborators, rat fVM cells were grafted alone or in combination with striatal (i.e., a major dopamine target area) or spinal cord (i.e., a non-target area) cells. The results showed that mixed mesencephalic and striatal suspensions gave rise to a greater area of dense dopaminergic innervation into the host striatum than grafts of fVM alone or fVM combined with spinal cord cells (Brundin et al., 1986). Similar results were obtained in subsequent studies, showing increased dopaminergic survival and more robust, long-lasting functional recovery in denervated rodents and monkeys after nigral-striatal co-grafts (Yurek et al., 1990; Costantini et al., 1994; Emgard-Mattson et al., 1997; Sortwell et al., 1998; Sladek et al., 2008). The effects of co-grafting fVM and striatal tissue in a PD patient were also explored and the results showed improved motor function and a significant increase in the uptake of fluoro-L-DOPA detected by positron emission tomography in the co-grafted putamen (i.e., grafts of fVM and lateral ganglionic eminence tissue) compared to the non-co-grafted side (i.e., a graft of fVM tissue alone) (Lee et al., 2003). The discovery of glial cell line derived-neurotrophic factor (GDNF), which is expressed in the rodent striatum, as the most potent neurotrophin that promotes dopaminergic survival quickly, sparked interest in this factor in neuroprotective and cell therapies for PD. Previous studies showed that GDNF rescues dopaminergic neurons in animal models of PD (Winkler et al., 1996). Different approaches to delivering GDNF into the brain, such as viral delivery, genetically modified cells, nanoparticles, or hydrogels, were used to increase the survival, maturation, and functional integration of transplanted dopaminergic cells (Moriarty et al., 2017; Gantner et al., 2020). Despite its potential, clinical translation of GDNF-related therapies was hampered by safety and efficacy concerns associated with its short half-life, low ability to cross the blood-brain barrier, and side effects derived from long-term delivery. Co-transplantation strategies have been proposed to achieve a sustained release of minimum effective levels of GDNF that could increase its therapeutic benefits. Initial studies showed that co-transplants of fVM and GDNF-secreting clones of a Schwann cell line enhanced the survival and fiber outgrowth from embryonic nigral cells grafted in 6-OHDA-lesioned rats (Wilby et al., 1999). Carotid body glomus cells are highly dopaminergic and produce GDNF, and their neuroprotective and neuroreparative effects have been demonstrated in rodent PD models (Villadiego et al., 2005) and trialed in PD patients (Arjona et al., 2003). Our group in collaboration with Toledo-Aral's team observed that the survival of dopaminergic neurons, the graft-derived dopaminergic reinnervation, and the performance of the cylinder test were improved in dopamine-depleted rats that received co-grafts of fVM and cell aggregates obtained from the carotid body compared to control groups that received fVM tissue alone (Rodríguez-Pallares et al., 2012). Other studies obtained similar results when different GDNF-producing cells such as fibroblasts, modified neural stem

cells or myoblasts were co-grafted with dopaminergic neurons (Ostenfeld et al., 2002; Jingzhong et al., 2005; Deng et al., 2013; Perez-Bouza et al., 2017). Transplantation of expanded neural precursor cells derived from the developing VM was also explored in animal models of PD (Armstrong et al., 2000; Rodríguez-Pallares et al., 2005). Co-grafting VM-derived precursors with cultured astrocytes resulted in almost complete behavioral recovery and a higher number of tyrosine hydroxylase (TH)-positive cells within the graft, with longer fiber length compared to control grafts (Song et al., 2018). Interestingly, astrocytes exhibited region-specific heterogeneity in their secretory profiles, and the beneficial effects on graft survival and function were more evident when VM-derived precursors were co-implanted in the same suspension with VM-derived astrocytes compared to co-transplants including cortex-derived astrocytes. These effects are possibly related to the establishment of a neurotrophic brain environment, as a result of an upregulated expression pattern of neurotrophic factors, trophic extracellular matrix proteins, and antioxidant factors in the striatum after co-transplantation. A reduction in the immune and inflammatory response was also suggested since an increase in the expression of anti-inflammatory markers (interleukin (IL)-1 and IL-10) and the downregulation of proinflammatory markers (IL-1 β and inducible nitric oxide synthase) were observed (Song et al., 2018; see for details in **Additional Table 1**).

As indicated above, the low survival of dopaminergic neurons during and after grafting is probably caused by a combination of factors beyond growth factor deprivation such as apoptosis, neuroinflammation, oxidative stress, hypoxia, and insufficient vascular supply, and immune rejection, suggesting that a pleiotropic therapy would be advantageous. In this regard, MSCs have broad neurotrophic, anti-inflammatory, and immunomodulatory properties, and have been proposed as a promising cell source for cell therapy in PD.

The Potential of Mesenchymal Stromal Cells for Cell Therapy

MSCs are multipotent cells that possess stem cell-like properties including self-renewal and the capacity to differentiate, under appropriate conditions, into several tissues of mesenchymal origin including cartilage, bone, and fat. MSCs were first isolated from bone marrow (BM) and defined as plastic-adherent and colony-forming unit fibroblastic cells. Although BM is the primary source, MSCs can also be isolated from many other sources including adipose tissue, umbilical cord Wharton's jelly, skin, dental pulp, peripheral blood, and perivascular niches, among others (Andrzejewska et al., 2019; Dabrowska et al., 2021). Interest in MSCs has increased rapidly in recent years due to their widespread availability in the body, high proliferation capacity, few ethical concerns, and low risk of tumor formation. Moreover, their migration and homing abilities (i.e., capacity to preferentially migrate toward damaged tissues), as well as their capacity to regulate the local environment through the release of immunomodulatory and trophic factors, make them an invaluable source for cell therapy (Fricova et al., 2020). MSCs can regulate mechanisms of both innate and adaptive immune responses, through the modulation of cellular responses and secretion of anti-inflammatory mediators. MSCs also participate in angiogenic processes by secreting different growth factors, cytokines, and chemokines. Moreover, the neuroprotective effects of MSCs on damaged or dying nerve cells, as discussed below, are particularly interesting. In addition to direct actions, different studies suggested that MSC-derived products, and particularly MSC-derived extracellular vesicles (MSC-EVs), elicited similar biological effects and have emerged as a powerful regenerative tool (Vilaça-Faria et al., 2019).

Mesenchymal Stromal Cells and Neural Repair

Therapies using MSCs rely on their capacity to modify damaged tissue microenvironments and enhance endogenous neural protection and regeneration. MSCs can secrete into the damaged area neurotrophic and anti-apoptotic factors, and cytokines such as insulin growth factor 1, vascular endothelial growth factor, brain-derived neurotrophic factor, fibroblast growth factor 2, and nerve growth factor, among others. MSCs can also promote neural repair through the production of anti-inflammatory cytokines such as IL-10 and transforming growth factor β and by decreasing pro-inflammatory cytokines such as IL-1 β , tumor necrosis factor- α , or interferon γ (Fricova et al., 2020). Moreover, there is evidence of the capacity of MSCs to counteract oxidative stress and enhance the antioxidant response (Angeloni et al., 2020). Extracellular matrix molecules produced by MSCs have also been demonstrated to promote neural cell attachment, growth, and axonal extension. Pre-treatment of MSCs under defined conditions (i.e., pre-conditioning) has been shown to modify the secretome of MSCs and improve their efficacy in animal models of different neurological disorders (Baez-Jurado et al., 2019; d'Angelo et al., 2020). Other strategies have been based on the use of genetically engineered MSCs as delivery vehicles to produce specific neurotrophic factors, such as brain-derived neurotrophic factor, or the modification of MSCs to improve their survival and capacity to migrate toward the lesion site, particularly relevant when systemic administrations are performed, or prevent their premature senescence (Ocansey et al., 2020). Significant attention has been paid to the therapeutic potential of MSC-EVs to promote neurite growth and remodeling, enhance neurogenesis and angiogenesis and exert neuroprotective effects in damaged neural tissue. Furthermore, MSC-EVs reduce brain inflammation by inhibiting astroglial and switching the polarization of macrophages and microglial cells from pro-inflammatory to pro-repair phenotypes. Treatment with MSC-EVs also reduced the levels of pro-inflammatory cytokines such as tumor necrosis factor- α ,

IL-1 β , and IL-6 and increased the levels of anti-inflammatory cytokines IL-4 and IL-10. Overall, MSCs have unique and valuable characteristics for the repair of the nervous system in various diseases including PD (d'Angelo et al., 2020).

Mesenchymal Stromal Cell-Based Therapies for Parkinson's Disease

Different studies suggest that MSCs can differentiate into neural progenitors from which cells resembling dopamine neurons (i.e., that express the dopaminergic marker TH) can be obtained (Thompson et al., 2019). Neuronal-primed MSCs or dopaminergic cells derived from MSCs have been transplanted in animal models of PD (Delcroix et al., 2011; Park et al., 2012a; Shetty et al., 2013; Wang et al., 2013). However, it remains unclear whether these cells can integrate into the host neural circuits and create new synapses with host neurons. Therefore, most studies have focused on their known ability to produce a wide range of factors. Transplantation of murine BM-MSC into the striatum or the substantia nigra in preclinical models of PD showed significant improvements in behavioral tests and preservation of TH-positive neurons in the substantia nigra and fibers in the striatum (Chen et al., 2017). Dopamine-depleted animals receiving intrastratial injections of human BM-MSCs grafts also showed neuroprotective effects (Blandini et al., 2010; Cova et al., 2010; Xiong et al., 2013). MSCs obtained from other sources such as adipose tissue or umbilical cord have also been tested with similar results (Schwerk et al., 2015; Bagheri-Mohammadi et al., 2019; Wang et al., 2020; Lian et al., 2021). The mechanisms underlying these improvements have been ascribed to increased levels of GDNF and brain-derived neurotrophic factor, reduction of apoptosis and inflammation, or neurogenic effects (Blandini et al., 2010; Cova et al., 2010; Lian et al., 2021). However, other studies showed modest or no improvement after MSCs grafting (Moloney et al., 2010; Sun et al., 2020). In addition to intracerebral grafting, other routes of administration have also been tested as suitable approaches in PD. Systemic administration of MSCs by intravenous or intracarotid injection induced a significant reduction in amphetamine-induced rotational behavior, preserved TH-positive cells in the substantia nigra and dopaminergic striatal terminals, and reduced astrocytic and microglial response in animal PD models (Chao et al., 2009; Park et al., 2012b; Inden et al., 2013; Cerri et al., 2015; Suzuki et al., 2015; Park and Chang, 2020). Although it has been suggested that systemic administered MSCs do not pass the capillary system of the lungs, other studies showed that MSCs have an intrinsic ability to target the brain and migrate to the lesioned substantia nigra after intravenous administration (Chao et al., 2009; Do et al., 2021). Other works have used intranasal infusion as a non-invasive method of administration. Although some authors reported that MSCs administered intranasally reached the brain parenchyma, migrated to lesioned areas, and survived (Danielyan et al., 2011; Salama et al., 2017), others indicated that MSCs could not be detected in the brain (Chartoff et al., 2011). To address these issues, several modification techniques have been proposed to efficiently guide their migration to target sites or improve focal adhesion, which may be of particular interest to improve their viability within the transplanted environment in co-grafting approaches (Ocansey et al., 2020). Another important aspect is the implantation of a sufficient number of MSCs to guarantee a therapeutic effect. Previous studies suggest that MSCs survive for only a short period after transplantation (Chartoff et al., 2011; Bossolasco et al., 2012). The low immunogenicity of MSCs does not ensure that they are fully immune privileged, and they are likely to provoke immune response despite their immunosuppressive properties (Camp et al., 2009). Different strategies have been tested to increase the survival of the transplanted MSCs such as cell encapsulation, a combination of MSCs with microcarriers that release therapeutic proteins, or co-grafting with OECs (Delcroix et al., 2011; Forouzandeh et al., 2021). Other studies have combined MSC transplantation with other therapeutic strategies such as levodopa treatment or exercise to obtain enhanced improvements (Cucarian et al., 2019; Jalali et al., 2021). Other mechanisms underlying the beneficial effects of MSCs have been proposed. Recent data suggest that MSCs can transfer mitochondria to damaged tissue, which seems particularly relevant in PD therapy due to the proposed central role of damaged mitochondria in dopaminergic neurodegeneration (Liu et al., 2014). Interestingly, it has recently been reported that MSC treatment alleviated locomotor deficits and rescued dopaminergic neurons by inhibiting neuroinflammation in a PD model through regulating intestinal microorganisms by cross talk between the brain and gut (Sun et al., 2022; see for details in **Additional Table 2**). As PD pathology involves widespread neurodegeneration beyond the nigrostriatal pathway, MSCs transplantation targeting extra basal ganglia structures could also represent a strategy to alleviate the non-motor symptoms of the disease (Pantcheva et al., 2015).

Delivery of neurotrophic factors by MSCs may also serve as a useful tool for the protection and functional stimulation of grafted dopaminergic cells from any source. In fact, co-grafting of fetal porcine neuroblasts and rat BM-MSCs promoted the survival of neuroblasts that when transplanted alone did not survive in the striatum of immunocompetent (i.e., non-immunosuppressed) denervated rats (Leveque et al., 2015). Interestingly, 50% of transplanted rats showed healthy grafts with TH-positive neurons at 120 days post-transplantation and motor recovery was also observed. Detailed analysis revealed a local control of the host immune response in the striatum that received co-grafts, highlighting the immunosuppressive properties of MSCs and their potential utility for the long-term survival of xenogeneic grafts (Leveque et al., 2015). Our group has recently demonstrated that the combination of murine BM-MSCs and fVM cells in the same suspension

significantly improves the survival and reinnervation ability of dopaminergic neurons transplanted in a rat 6-OHDA model of PD. However, it is essential to control tightly the density of the transplanted cells or concentration of MSC-derived products, since these beneficial effects are only observed when fVM tissue is combined with a low number of MSCs (Rodriguez-Pallares et al., 2021; **Additional Table 1**). Interestingly, a conditioned medium derived from MSC (MSC-CM) containing the entire secretome also enhances the survival of dopaminergic grafts (Shintani et al., 2007), suggesting that the therapeutic effects of MSCs are not only based on direct cell-to-cell interactions but that paracrine signaling seems to be a primary mechanism (Mendes-Pinheiro et al., 2019; Fricova et al., 2020).

As previously indicated, several mechanisms could be responsible for these effects. Our group observed that classical pathways involved in trophic factor-mediated signaling, such as MAPK/ERK and PI3K/Akt pathways, were active in dopaminergic cultures treated with MSC-CM. However, lipid removal decreased the viability and reduced the neuroprotective effects of MSC-CM on pure populations of dopaminergic cells, primary mesencephalic cultures (i.e., neuron-glia mixed), and human iPSC-derived neurons (Parga et al., 2018), suggesting that other factors could also be relevant. We demonstrated for the first time that prostaglandin E₂, through prostaglandin E₂ receptor 2, was largely responsible for the positive effects of the MSC-CM on dopaminergic survival and the cAMP-PKA pathway, used by many prostaglandins to modulate neuroprotection, was active in dopaminergic cultures (Parga et al., 2018).

Based on these promising results, several groups have conducted clinical trials using autologous or allogenic MSCs isolated from various sources for PD treatment (Fricova et al., 2020). In a pilot, open-label study in 2010, autologous BM-MSCs were transplanted unilaterally into the subthalamic ventricular zone in seven PD patients. This preliminary study suggests the safety of this novel therapeutic approach and its potential beneficial effects, since three patients showed improved symptoms (Venkataramana et al., 2010). Two years later, the same research group conducted another open-label study using allogenic BM-MSCs implanted bilaterally into the same area with similar results (Venkataramana et al., 2012). Currently, several clinical trials are in progress with a highly variable setup (ClinicalTrials.gov identifiers NCT02611167; NCT03550183; NCT04506073; NCT04928287; NCT04995081), including a study of co-transplantation of MSCs and MSC-derived neural stem cells in PD patients (ClinicalTrials.gov identifier NCT03684122). The experience gained in previous trials should guide the future directions and emphasize the need for further studies to understand the mechanisms underlying the therapeutic effects of MSCs and standardize experimental designs and protocols to achieve reliable and effective cell-based treatments for PD.

Conclusions and Future Perspectives

Over the decades, cell-based therapies have shown their potential in the treatment of PD. Although prematurely conducted trials in the past have cast doubt on the efficacy of these emerging approaches, human fVM tissue continues to be the gold standard and has undoubtedly paved the way for considering the clinical translation of transplantation of dopaminergic neurons obtained from different sources. The number of healthy dopaminergic cells needed for functional improvements in PD patients is around 40,000–80,000 neurons, which requires readily available cells that contribute to permit the large-scale application of these approaches. Stem cells offer a clear advantage in this regard, and significant progress in obtaining sufficient dopaminergic cells from different expandable sources has been achieved in recent years. However, cell therapy for PD has underlying problems that affect transplanted cells regardless of the source of dopaminergic neurons. Although further studies are necessary, combinations of cell-based strategies, such as the co-transplantation approaches addressed in this review, or combination of cells with pharmacological treatments, deep brain stimulation, or potential new immunological therapies could improve the current results and help to slow or even stop the progression of the disease. Genetic manipulation of the cells may also be another beneficial strategy that could contribute to improving the survival, integration, and functionality of grafted cells. Emerging approaches in neural repair involve the development and application of tissue engineering techniques, such as the use of biomaterial or cellular scaffolds, or patient-specific constructs, that could influence the behavior of native or transplanted cells and promote survival, differentiation, organization, and reconstruction of degenerated circuitry. Further research is necessary to understand the mechanisms underlying the functional recovery of successful transplants and to achieve greater benefits for the millions of patients worldwide affected by PD.

Author contributions: Manuscript conception and writing: JRP and JLLG; manuscript correction and editing: JAP and MGG. All the authors approved the final version of the manuscript.

Conflicts of interest: The authors declare no conflicts of interest.

Availability of data and materials: All data generated or analyzed during this study are included in this published article and its supplementary information files.

Open access statement: This is an open access journal, and articles are distributed under the terms of the Creative Commons AttributionNonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the

identical terms.

Additional files:

Additional Table 1: *Co-grafting-based strategies in Parkinson's disease.*

Additional Table 2: *Mesenchymal stromal cell-based strategies in Parkinson's disease.*

References

- Agrawal AK, Shukla S, Chaturvedi RK, Seth K, Srivastava N, Ahmad A, Seth PK (2004) Olfactory ensheathing cell transplantation restores functional deficits in a rat model of Parkinson's disease: a cotransplantation approach with fetal ventral mesencephalic cells. *Neurobiol Dis* 16:516-526.
- Aguiar AJ, Bjorklund A, Stenevi U, Carlstedt T (1984) Fetal mesencephalic neurons survive and extend long axons across peripheral nervous system grafts inserted into the adult rat striatum. *Neurosci Lett* 45:53-58.
- Andrzejewska A, Lukomska B, Janowski M (2019) Concise review: mesenchymal stem cells: From roots to boost. *Stem Cells* 37:855-864.
- Angeloni C, Gatti M, Prata C, Hrelia S, Maraldi T (2020) Role of mesenchymal stem cells in counteracting oxidative stress-related neurodegeneration. *Int J Mol Sci* 21:3299.
- Arjona V, Minguez-Castellanos A, Montoro RJ, Ortega A, Escamilla F, Toledo-Aral JJ, Pardal R, Mendez-Ferrer S, Martin JM, Perez M, Katati MJ, Valencia E, Garcia T, Lopez-Barneo J (2003) Autotransplantation of human carotid body cell aggregates for treatment of parkinson's disease. *Neurosurgery* 53:321-330.
- Armstrong MJ, Okun MS (2020) Diagnosis and treatment of Parkinson disease: a review. *JAMA* 323:548-560.
- Armstrong RJ, Watts C, Svendsen CN, Dunnett SB, Rosser AE (2000) Survival, neuronal differentiation, and fiber outgrowth of propagated human neural precursor grafts in an animal model of huntington's disease. *Cell Transplant* 9:55-64.
- Baez-Jurado E, Hidalgo-Lanussa O, Barrera-Bailon B, Sahebkar A, Ashraf GM, Echeverria V, Barreto GE (2019) Secretome of mesenchymal stem cells and its potential protective effects on brain pathologies. *Mol Neurobiol* 56:6902-6927.
- Bagheri-Mohammadi S, Alani B, Karimian M, Moradian-Tehrani R, Nouredini M (2019) Intranasal administration of endometrial mesenchymal stem cells as a suitable approach for Parkinson's disease therapy. *Mol Biol Rep* 46:4293-4302.
- Barker RA, Dunnett SB, Faissner A, Fawcett JW (1996) The time course of loss of dopaminergic neurons and the gliotic reaction surrounding grafts of embryonic mesencephalon to the striatum. *Exp Neurol* 141:79-93.
- Barker RA, TRANSEURO consortium (2019) Designing stem-cell-based dopamine cell replacement trials for Parkinson's disease. *Nat Med* 25:1045-1053.
- Barker RA, Bjorklund A, Gash DM, Whone A, Van Laar A, Kordower JH, Bankiewicz K, Kiebertz K, Saarma M, Booms S, Huttunen HJ, Kells AP, Fiandaca MS, Stoessl AJ, Eidelberg D, Federoff H, Voutilainen MH, Dexter DT, Eberling J, Brundin P, et al (2020) GDNF and Parkinson's disease: where next? A summary from a recent workshop. *J Parkinsons Dis* 10:875-891.
- Bjorklund A, Schmidt RH, Stenevi U (1980) Functional reinnervation of the neostriatum in the adult rat by use of intraparenchymal grafting of dissociated cell suspensions from the substantia nigra. *Cell Tissue Res* 212:39-45.
- Bjorklund A, Stenevi U, Schmidt RH, Dunnett SB, Gage FH (1983) Intracerebral grafting of neuronal cell suspensions. II. survival and growth of nigral cell suspensions implanted in different brain sites. *Acta Physiol Scand Suppl* 522:9-18.
- Bjorklund A, Lindvall O (2017) Replacing dopamine neurons in Parkinson's disease: how did it happen? *J Parkinsons Dis* 7:S21-31.
- Blandini F, Cova L, Armentero MT, Zennaro E, Levandis G, Bossolasco P, Calzarossa C, Mellone M, Giuseppe B, Dellilieri GL, Polli E, Nappi G, Silani V (2010) Transplantation of undifferentiated human mesenchymal stem cells protects against 6-hydroxydopamine neurotoxicity in the rat. *Cell Transplant* 19:203-217.
- Bossolasco P, Cova L, Levandis G, Diana V, Cerri S, Lambertenghi Dellilieri G, Polli E, Silani V, Blandini F, Armentero MT (2012) Noninvasive near-infrared live imaging of human adult mesenchymal stem cells transplanted in a rodent model of Parkinson's disease. *Int J Nanomedicine* 7:435-447.
- Brundin P, Nilsson OG, Strecker RE, Lindvall O, Asted B, Bjorklund A (1986) Behavioural effects of human fetal dopamine neurons grafted in a rat model of Parkinson's disease. *Exp Brain Res* 65:235-240.
- Brundin P, Karlsson J, Emgard M, Schierle GS, Hansson O, Petersen A, Castilho RF (2000) Improving the survival of grafted dopaminergic neurons: a review over current approaches. *Cell Transplant* 9:179-195.
- Camp DM, Loeffler DA, Farrah DM, Borneman JN, LeWitt PA (2009) Cellular immune response to intrastrially implanted allogeneic bone marrow stromal cells in a rat model of Parkinson's disease. *J Neuroinflammation* 6:17-17.
- Cerri S, Greco R, Levandis G, Ghezzi C, Mangione AS, Fuzzati-Armentero MT, Bonizzi A, Avanzini MA, Maccario R, Blandini F (2015) Intracarotid infusion of mesenchymal stem cells in an animal model of Parkinson's disease, focusing on cell distribution and neuroprotective and behavioral effects. *Stem Cells Transl Med* 4:1073-1085.
- Chao YX, He BP, Tay SS (2009) Mesenchymal stem cell transplantation attenuates blood brain barrier damage and neuroinflammation and protects dopaminergic neurons against MPTP toxicity in the substantia nigra in a model of Parkinson's disease. *J Neuroimmunol* 216:39-50.
- Chartoff EH, Damez-Werno D, Sonntag KC, Hassinger L, Kaufmann DE, Peterson J, McPhie D, Cataldo AM, Cohen BM (2011) Detection of intranasally delivered bone marrow-derived mesenchymal stromal cells in the lesioned mouse brain: A cautionary report. *Stem Cells Int* 2011:586586.
- Chen D, Fu W, Zhuang W, Lv C, Li F, Wang X (2017) Therapeutic effects of intranigral transplantation of mesenchymal stem cells in rat models of Parkinson's disease. *J Neurosci Res* 95:907-917.
- Collier TJ, Elsworth JD, Taylor JR, Sladek JR, Roth RH, Redmond DE (1994) Peripheral nerve-dopamine neuron co-grafts in MPTP-treated monkeys: augmentation of tyrosine hydroxylase-positive fiber staining and dopamine content in host systems. *Neuroscience* 61:875-889.
- Costantini LC, Voza BM, Snyder-Keller AM (1994) Enhanced efficacy of nigral-striatal cotransplants in bilaterally dopamine-depleted rats: An anatomical and behavioral analysis. *Exp Neurol* 127:219-231.
- Cova L, Armentero MT, Zennaro E, Calzarossa C, Bossolasco P, Busca G, Lambertenghi Dellilieri G, Polli E, Nappi G, Silani V, Blandini F (2010) Multiple neurogenic and neurorescue effects of human mesenchymal stem cell after transplantation in an experimental model of Parkinson's disease. *Brain Res* 1311:12-27.
- Cucarian JD, Berrio JP, Rodrigues C, Zancan M, Wink MR, de Oliveira A (2019) Physical exercise and human adipose-derived mesenchymal stem cells ameliorate motor disturbances in a male rat model of Parkinson's disease. *J Neurosci Res* 97:1095-1109.
- Dabrowska S, Andrzejewska A, Janowski M, Lukomska B (2021) Immunomodulatory and regenerative effects of mesenchymal stem cells and extracellular vesicles: Therapeutic outlook for inflammatory and degenerative diseases. *Front Immunol* 11:591065.
- d'Angelo M, Cimini A, Castelli V (2020) Insights into the effects of mesenchymal stem cell-derived secretome in Parkinson's disease. *Int J Mol Sci* 21:10.3390/ijms21155241.
- Danielyan L, Schafer R, von Ameln-Mayerhofer A, Bernhard F, Verleysdonk S, Buadze M, Lourhmati A, Klopfer T, Schaumann F, Schmid B, Koehle C, Proksch B, Weissert R, Reichardt HM, van den Brandt J, Buniatian GH, Schwab M, Gleiter CH, Frey WH (2011) Therapeutic efficacy of intranasally delivered mesenchymal stem cells in a rat model of Parkinson disease. *Rejuvenation Res* 14:3-16.
- Date I, Felten SY, Olschowka JA, Felten DL (1990) Limited recovery of striatal dopaminergic fibers by adrenal medullary grafts in MPTP-treated aging mice. *Exp Neurol* 107:197-207.
- Delcroix GJ, Garbayo E, Sindji L, Thomas O, Vanpouille-Box C, Schiller PC, Montero-Menei CN (2011) The therapeutic potential of human multipotent mesenchymal stromal cells combined with pharmacologically active microcarriers transplanted in hemi-Parkinsonian rats. *Biomaterials* 32:1560-1573.
- Deng X, Liang Y, Lu H, Yang Z, Liu R, Wang J, Song X, Long J, Li Y, Lei D, Feng Z (2013) Co-transplantation of GDNF-overexpressing neural stem cells and fetal dopaminergic neurons mitigates motor symptoms in a rat model of Parkinson's disease. *PLoS One* 8:e80880.
- Denis-Donini S, Estenoz M (1988) Interneurons versus efferent neurons: Heterogeneity in their neurite outgrowth response to glia from several brain regions. *Dev Biol* 130:237-249.
- Dewar D, Bentley D, Barnett SC (2007) Implantation of pure cultured olfactory ensheathing cells in an animal model of Parkinsonism. *Acta Neurochir (Wien)* 149:407-414.
- Do AD, Kurniawati I, Hsieh CL, Wong TT, Lin YL, Sung SY (2021) Application of mesenchymal stem cells in targeted delivery to the brain: Potential and challenges of the extracellular vesicle-based approach for brain tumor treatment. *Int J Mol Sci* 22:10.3390/ijms22011187.

- Emgard-Mattson M, Karlsson J, Nakao N, Brundin P (1997) Addition of lateral ganglionic eminence to rat mesencephalic grafts affects fiber outgrowth but does not enhance function. *Cell Transplant* 6:277-286.
- Fan Y, Winanto, Ng SY (2020) Replacing what's lost: a new era of stem cell therapy for Parkinson's disease. *Transl Neurodegener* 9:2.
- Forouzandeh M, Bigdeli MR, Mostafavi H, Nadri S, Eskandari M (2021) Therapeutic potentials of human microfluidic encapsulated conjunctival mesenchymal stem cells on the rat model of Parkinson's disease. *Exp Mol Pathol* 123:104703.
- Freed CR, Greene PE, Breeze RE, Tsai WY, DuMouchel W, Kao R, Dillon S, Winfield H, Culver S, Trojanowski JQ, Eidelberg D, Fahn S (2001) Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med* 344:710-719.
- Freed WJ, Morihisa JM, Spoor E, Hoffer BJ, Olson L, Seiger A, Wyatt RJ (1981) Transplanted adrenal chromaffin cells in rat brain reduce lesion-induced rotational behaviour. *Nature* 292:351-352.
- Freed WJ, Poltorak M, Becker JB (1990) Intracerebral adrenal medulla grafts: a review. *Exp Neurol* 110:139-166.
- Fricova D, Korchak JA, Zubair AC (2020) Challenges and translational considerations of mesenchymal stem/stromal cell therapy for Parkinson's disease. *NPJ Regen Med* 5:20.
- Gantner CW, de Luzy IR, Kauhausen JA, Moriarty N, Niclis JC, Bye CR, Penna V, Hunt CPJ, Ermine CM, Pouton CW, Kirik D, Thompson LH, Parish CL (2020) Viral delivery of GDNF promotes functional integration of human stem cell grafts in Parkinson's disease. *Cell Stem Cell* 26:511-526.
- Hagell P, Piccini P, Bjorklund A, Brundin P, Rehncrona S, Widner H, Crabb L, Pavese N, Oertel WH, Quinn N, Brooks DJ, Lindvall O (2002) Dyskinesias following neural transplantation in Parkinson's disease. *Nat Neurosci* 5:627-628.
- Hallett PJ, Cooper O, Sadi D, Robertson H, Mendez I, Isacson O (2014) Long-term health of dopaminergic neuron transplants in Parkinson's disease patients. *Cell Rep* 7:1755-1761.
- Hiller BM, Marmion DJ, Thompson CA, Elliott NA, Federoff H, Brundin P, Mattis VB, McMahon CW, Kordower JH (2022) Optimizing maturity and dose of iPSC-derived dopamine progenitor cell therapy for Parkinson's disease. *NPJ Regen Med* 7:24.
- Inden M, Takata K, Nishimura K, Kitamura Y, Ashihara E, Yoshimoto K, Ariga H, Honmou O, Shimohama S (2013) Therapeutic effects of human mesenchymal and hematopoietic stem cells on rotenone-treated Parkinsonian mice. *J Neurosci Res* 91:62-72.
- Jalali MS, Saki G, Farbood Y, Azandeh SS, Mansouri E, Ghasemi Dehcheshmeh M, Sarkaki A (2021) Therapeutic effects of Wharton's jelly-derived mesenchymal stromal cells on behaviors, EEG changes and NGF-1 in rat model of the Parkinson's disease. *J Chem Neuroanat* 113:101921.
- Jang SE, Qiu L, Chan LL, Tan EK, Zeng L (2020) Current status of stem cell-derived therapies for Parkinson's disease: From cell assessment and imaging modalities to clinical trials. *Front Neurosci* 14:558532.
- Jingzhong Z, Hui Y, Deyi D, Chunli D, Chunli Z, Xiaohong S, Jinlu Z, Qunyuan X (2005) Long-term therapeutic effects on Parkinsonian rats of intrastriatal co-grafts with genetically engineered fibroblasts expressing tyrosine hydroxylase and glial cell line-derived neurotrophic factor. *Int J Neurosci* 115:769-779.
- Johansson S, Lee IH, Olson L, Spenger C (2005) Olfactory ensheathing glial co-grafts improve functional recovery in rats with 6-OHDA lesions. *Brain* 128:2961-2976.
- Kim TW, Koo SY, Studer L (2020) Pluripotent stem cell therapies for Parkinson disease: Present challenges and future opportunities. *Front Cell Dev Biol* 8:729.
- Lee CC, Lin SZ, Wang Y, Lin JJ, Liu JY, Chen GJ, Chiang YH, Liu JC, Zhou FC (2003) First human ventral mesencephalon and striatum cografting in a Parkinson patient. *Acta Neurochir Suppl* 87:159-162.
- Leveque X, Mathieux E, Nerriere-Daguin V, Thinard R, Kermerrec L, Durand T, Haudebourg T, Vanhove B, Lescaudron L, Neveu I, Naveilhan P (2015) Local control of the host immune response performed with mesenchymal stem cells: Perspectives for functional intracerebral xenotransplantation. *J Cell Mol Med* 19:124-134.
- Li JY, Li W (2021) Postmortem studies of fetal grafts in Parkinson's disease: what lessons have we learned? *Front Cell Dev Biol* 9:666675.
- Lian C, Huang Q, Zhong X, He Z, Liu B, Zeng H, Xu N, Yang Z, Liao C, Fu Z, Guo H (2021) Pentraxin 3 secreted by human adipose-derived stem cells promotes dopaminergic neuron repair in Parkinson's disease via the inhibition of apoptosis. *Faseb J* 35:e21748.
- Lindvall O, Brundin P, Widner H, Rehncrona S, Gustavii B, Frackowiak R, Leenders KL, Sawle G, Rothwell JC, Marsden CD (1990) Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. *Science* 247:574-577.
- Liu K, Ji K, Guo L, Wu W, Lu H, Shan P, Yan C (2014) Mesenchymal stem cells rescue injured endothelial cells in an in vitro ischemia-reperfusion model via tunneling nanotube like structure-mediated mitochondrial transfer. *Microvasc Res* 92:10-18.
- Lopez-Lopez A, Labandeira CM, Labandeira-Garcia JL, Muñoz A (2020) Rho kinase inhibitor fasudil reduces L-DOPA-induced dyskinesia in a rat model of Parkinson's disease. *Br J Pharmacol* 177:5622-5641.
- Ma C, Zhang W, Cao M (2021) Role of the peripheral nervous system in PD pathology, diagnosis, and treatment. *Front Neurosci* 15:598457.
- Mendes-Pinheiro B, Anjo SI, Manadas B, Da Silva JD, Marote A, Behie LA, Teixeira FG, Salgado AJ (2019) Bone marrow mesenchymal stem cells' secretome exerts neuroprotective effects in a Parkinson's disease rat model. *Front Bioeng Biotechnol* 7:294.
- Moloney TC, Rooney GE, Barry FP, Howard L, Dowd E (2010) Potential of rat bone marrow-derived mesenchymal stem cells as vehicles for delivery of neurotrophins to the Parkinsonian rat brain. *Brain Res* 1359:33-43.
- Moriarty N, Pandit A, Dowd E (2017) Encapsulation of primary dopaminergic neurons in a GDNF-loaded collagen hydrogel increases their survival, re-innervation and function after intra-striatal transplantation. *Sci Rep* 7:16033.
- Muñoz A, Lopez-Lopez A, Labandeira CM, Labandeira-Garcia JL (2020) Interactions between the serotonergic and other neurotransmitter systems in the basal ganglia: role in Parkinson's disease and adverse effects of L-DOPA. *Front Neuroanat* 14:26.
- Ocansey DKW, Pei B, Yan Y, Qian H, Zhang X, Xu W, Mao F (2020) Improved therapeutics of modified mesenchymal stem cells: An update. *J Transl Med* 18:42.
- Ostenfeld T, Tai YT, Martin P, Deglon N, Aebischer P, Svendsen CN (2002) Neurospheres modified to produce glial cell line-derived neurotrophic factor increase the survival of transplanted dopamine neurons. *J Neurosci Res* 69:955-965.
- Pantcheva P, Reyes S, Hoover J, Kaelber S, Borlongan CV (2015) Treating non-motor symptoms of Parkinson's disease with transplantation of stem cells. *Expert Rev Neurother* 15:1231-1240.
- Parga JA, Garcia-Garrote M, Martinez S, Raya A, Labandeira-Garcia JL, Rodriguez-Pallares J (2018) Prostaglandin EP2 receptors mediate mesenchymal stromal cell-neuroprotective effects on dopaminergic neurons. *Mol Neurobiol* 55:4763-4776.
- Park H, Chang KA (2020) Therapeutic potential of repeated intravenous transplantation of human adipose-derived stem cells in subchronic MPTP-induced Parkinson's disease mouse model. *Int J Mol Sci* 21:8129.
- Park HJ, Shin JY, Lee BR, Kim HO, Lee PH (2012b) Mesenchymal stem cells augment neurogenesis in the subventricular zone and enhance differentiation of neural precursor cells into dopaminergic neurons in the substantia nigra of a Parkinsonian model. *Cell Transplant* 21:1629-1640.
- Park S, Kim E, Koh SE, Maeng S, Lee WD, Lim J, Shim I, Lee YJ (2012a) Dopaminergic differentiation of neural progenitors derived from placental mesenchymal stem cells in the brains of Parkinson's disease model rats and alleviation of asymmetric rotational behavior. *Brain Res* 1466:158-166.
- Parmar M, Grealish S, Henchcliffe C (2020) The future of stem cell therapies for Parkinson disease. *Nat Rev Neurosci* 21:103-115.
- Perez-Bouza A, Di Santo S, Seiler S, Meyer M, Anderegg L, Huber A, Guzman R, Widner HR (2017) Simultaneous transplantation of fetal ventral mesencephalic tissue and encapsulated genetically modified cells releasing GDNF in a hemi-Parkinsonian rat model of Parkinson's disease. *Cell Transplant* 26:1572-1581.
- Piao J, Zabierowski S, Dubose BN, Hill EJ, Navare M, Claros N, Rosen S, Ramnarine K, Horn C, Fredrickson C, Wong K, Safford B, Kriks S, El Maarouf A, Rutishauser U, Henchcliffe C, Wang Y, Riviere I, Mann S, Bermudez V, et al (2021) Preclinical efficacy and safety of a human embryonic stem cell-derived midbrain dopamine progenitor product, MSK-DA01. *Cell Stem Cell* 28:217-229.
- Piccini P, Brooks DJ, Bjorklund A, Gunn RN, Grasby PM, Rimoldi O, Brundin P, Hagell P, Rehncrona S, Widner H, Lindvall O (1999) Dopamine release from nigral transplants visualized in vivo in a Parkinson's patient. *Nat Neurosci* 2:1137-1140.
- Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkman J, Schrag AE, Lang AE (2017) Parkinson disease. *Nat Rev Dis Primers* 3:17013.
- Politis M, Wu K, Loane C, Quinn NP, Brooks DJ, Rehncrona S, Bjorklund A, Lindvall O, Piccini P (2010) Serotonergic neurons mediate dyskinesia side effects in Parkinson's patients with neural transplants. *Sci Transl Med* 2:38ra46.

- Rahimpour S, Zhang SC, Vitek JL, Mitchell KT, Turner DA (2022) Comparative efficacy of surgical approaches to disease modification in Parkinson disease. *NPJ Parkinsons Dis* 8:33.
- Rodríguez-Pallares J, Parga JA, Rey P, Guerra MJ, Labandeira-García JL (2005) Expanded mesencephalic precursors develop into grafts of densely packed dopaminergic neurons that reinnervate the surrounding striatum and induce functional responses in the striatal neurons. *Synapse* 58:13-22.
- Rodríguez-Pallares J, Joglar B, Muñoz-Manchado AB, Villadiego J, Toledo-Aral JJ, Labandeira-García JL (2012) Cograftering of carotid body cells improves the long-term survival, fiber outgrowth and functional effects of grafted dopaminergic neurons. *Regen Med* 7:309-322.
- Rodríguez-Pallares J, García-Garrote M, Parga JA, Labandeira-García JL (2021) Dose-dependent effect of mesenchymal stromal cells co-grafted with dopaminergic neurons in a Parkinson's disease rat model. *J Cell Mol Med* 25:9884-9889.
- Salama M, Sobh M, Emam M, Abdalla A, Sabry D, El-Gamal M, Lotfy A, El-Husseiny M, Sobh M, Shalash A, Mohamed WM (2017) Effect of intranasal stem cell administration on the nigrostriatal system in a mouse model of Parkinson's disease. *Exp Ther Med* 13:976-982.
- Schweitzer JS, Song B, Herrington TM, Park TY, Lee N, Ko S, Jeon J, Cha Y, Kim K, Li Q, Henchcliffe C, Kaplitt M, Neff C, Rapalino O, Seo H, Lee IH, Kim J, Kim T, Petsko GA, Ritz J, et al. (2020) Personalized iPSC-derived dopamine progenitor cells for Parkinson's disease. *N Engl J Med* 382:1926-1932.
- Schwerk A, Altschuler J, Roch M, Gossen M, Winter C, Berg J, Kurtz A, Akyuz L, Steiner B (2015) Adipose-derived human mesenchymal stem cells induce long-term neurogenic and anti-inflammatory effects and improve cognitive but not motor performance in a rat model of Parkinson's disease. *Regen Med* 10:431-446.
- Seiger A, Olson L (1975) Brain tissue transplanted to the anterior chamber of the eye: 3. substitution of lacking central noradrenaline input by host iris sympathetic fibers in the isolated cerebral cortex developed in oculo. *Cell Tissue Res* 159:325-338.
- Shetty P, Thakur AM, Viswanathan C (2013) Dopaminergic cells, derived from a high efficiency differentiation protocol from umbilical cord derived mesenchymal stem cells, alleviate symptoms in a Parkinson's disease rodent model. *Cell Biol Int* 37:167-180.
- Shintani A, Nakao N, Kakishita K, Itakura T (2007) Protection of dopamine neurons by bone marrow stromal cells. *Brain Res* 1186:48-55.
- Shukla S, Chaturvedi RK, Seth K, Roy NS, Agrawal AK (2009) Enhanced survival and function of neural stem cells-derived dopaminergic neurons under influence of olfactory ensheathing cells in Parkinsonian rats. *J Neurochem* 109:436-451.
- Sladek JR, Bjugstad KB, Collier TJ, Bundock EA, Blanchard BC, Elsworth JD, Roth RH, Redmond DE (2008) Embryonic substantia nigra grafts show directional outgrowth to cogenerated striatal grafts and potential for pathway reconstruction in nonhuman primate. *Cell Transplant* 17:427-444.
- Song JJ, Oh SM, Kwon OC, Wulansari N, Lee HS, Chang MY, Lee E, Sun W, Lee SE, Chang S, An H, Lee CJ, Lee SH (2018) Cograftering astrocytes improves cell therapeutic outcomes in a Parkinson's disease model. *J Clin Invest* 128:463-482.
- Sortwell CE, Collier TJ, Sladek JR (1998) Co-grafted embryonic striatum increases the survival of grafted embryonic dopamine neurons. *J Comp Neurol* 399:530-540.
- Sortwell CE, Pitzer MR, Collier TJ (2000) Time course of apoptotic cell death within mesencephalic cell suspension grafts: Implications for improving grafted dopamine neuron survival. *Exp Neurol* 165:268-277.
- Studer L, Tabar V (2020) Parkinson's disease grafts benefit from well-timed growth factor. *Nature* 582:39-40.
- Sun S, Zhang Q, Li M, Gao P, Huang K, Beejadhursing R, Jiang W, Lei T, Zhu M, Shu K (2020) GDNF promotes survival and therapeutic efficacy of human adipose-derived mesenchymal stem cells in a mouse model of Parkinson's disease. *Cell Transplant* 29:963689720908512.
- Sun Z, Gu P, Xu H, Zhao W, Zhou Y, Zhou L, Zhang Z, Wang W, Han R, Chai X, An S (2022) Human umbilical cord mesenchymal stem cells improve locomotor function in Parkinson's disease mouse model through regulating intestinal microorganisms. *Front Cell Dev Biol* 9:808905.
- Suzuki S, Kawamata J, Iwahara N, Matsumura A, Hisahara S, Matsushita T, Sasaki M, Honmou O, Shimohama S (2015) Intravenous mesenchymal stem cell administration exhibits therapeutic effects against 6-hydroxydopamine-induced dopaminergic neurodegeneration and glial activation in rats. *Neurosci Lett* 584:276-281.
- Takahashi J (2021) Clinical trial for Parkinson's disease gets a green light in the US. *Cell Stem Cell* 28:182-183.
- Thompson R, Casali C, Chan C (2019) Forskolin and IBMX induce neural transdifferentiation of MSCs through downregulation of the NRSF. *Sci Rep* 9:2969.
- Timmer M, Müller-Ostermeyer F, Kloth V, Winkler C, Grothe C, Nikkha G (2004) Enhanced survival, reinnervation, and functional recovery of intrastriatal dopamine grafts co-transplanted with schwann cells overexpressing high molecular weight FGF-2 isoforms. *Exp Neurol* 187:118-136.
- van Horne CG, Stromberg I, Young D, Olson L, Hoffer B (1991) Functional enhancement of intrastriatal dopamine-containing grafts by the co-transplantation of sciatic nerve tissue in 6-hydroxydopamine-lesioned rats. *Exp Neurol* 113:143-154.
- Venkataramana NK, Kumar SK, Balaraju S, Radhakrishnan RC, Bansal A, Dixit A, Rao DK, Das M, Jan M, Gupta PK, Totey SM (2010) Open-labeled study of unilateral autologous bone-marrow-derived mesenchymal stem cell transplantation in Parkinson's disease. *Transl Res* 155:62-70.
- Venkataramana NK, Pal R, Rao SA, Naik AL, Jan M, Nair R, Sanjeev CC, Kamble RB, Murthy DP, Chaitanya K (2012) Bilateral transplantation of allogenic adult human bone marrow-derived mesenchymal stem cells into the subventricular zone of Parkinson's disease: a pilot clinical study. *Stem Cells Int* 2012:931902.
- Vilaça-Faria H, Salgado AJ, Teixeira FG (2019) Mesenchymal stem cells-derived exosomes: A new possible therapeutic strategy for Parkinson's disease? *Cells* 8:118.
- Villadiego J, Mendez-Ferrer S, Valdes-Sanchez T, Silos-Santiago I, Farinas I, Lopez-Barneo J, Toledo-Aral JJ (2005) Selective glial cell line-derived neurotrophic factor production in adult dopaminergic carotid body cells in situ and after intrastriatal transplantation. *J Neurosci* 25:4091-4098.
- Wang Y, Yang J, Li H, Wang X, Zhu L, Fan M, Wang X (2013) Hypoxia promotes dopaminergic differentiation of mesenchymal stem cells and shows benefits for transplantation in a rat model of Parkinson's disease. *PLoS One* 8:e54296.
- Wang YL, Liu XS, Wang SS, Xue P, Zeng ZL, Yang XP, Zhang SM, Zheng W, Hua L, Li JF, Wang HT, Guo S (2020) Curcumin-activated mesenchymal stem cells derived from human umbilical cord and their effects on MPTP-mouse model of Parkinson's disease: a new biological therapy for Parkinson's disease. *Stem Cells Int* 2020:4636397.
- Weng SJ, Li IH, Huang YS, Chueh SH, Chou TK, Huang SY, Shiue CY, Cheng CY, Ma KH (2017) KA-bridged transplantation of mesencephalic tissue and olfactory ensheathing cells in a Parkinsonian rat model. *J Tissue Eng Regen Med* 11:2024-2033.
- Weng SJ, Chen CF, Huang YS, Chiu CH, Wu SC, Lin CY, Chueh SH, Cheng CY, Ma KH (2020) Olfactory ensheathing cells improve the survival of porcine neural xenografts in a Parkinsonian rat model. *Xenotransplantation* 27:e12569.
- Wilby MJ, Sinclair SR, Muir EM, Zietlow R, Adcock KH, Horellou P, Rogers JH, Dunnett SB, Fawcett JW (1999) A glial cell line-derived neurotrophic factor-secreting clone of the schwann cell line SCTM41 enhances survival and fiber outgrowth from embryonic nigral neurons grafted to the striatum and to the lesioned substantia nigra. *J Neurosci* 19:2301-2312.
- Winkler C, Sauer H, Lee CS, Bjorklund A (1996) Short-term GDNF treatment provides long-term rescue of lesioned nigral dopaminergic neurons in a rat model of Parkinson's disease. *J Neurosci* 16:7206-7215.
- Xiong M, Tao Y, Gao Q, Feng B, Yan W, Zhou Y, Kotsonis TA, Yuan T, You Z, Wu Z, Xi J, Haberman A, Graham J, Block J, Zhou W, Chen Y, Zhang SC (2021) Human stem cell-derived neurons repair circuits and restore neural function. *Cell Stem Cell* 28:112-126.
- Xiong N, Yang H, Liu L, Xiong J, Zhang Z, Zhang X, Jia M, Huang J, Zhang Z, Mohamed AA, Lin Z, Wang T (2013) bFGF promotes the differentiation and effectiveness of human bone marrow mesenchymal stem cells in a rotenone model for Parkinson's disease. *Environ Toxicol Pharmacol* 36:411-422.
- Yurek DM, Collier TJ, Sladek JR (1990) Embryonic mesencephalic and striatal co-grafts: Development of grafted dopamine neurons and functional recovery. *Exp Neurol* 109:191-199.

Additional Table 1: Co-grafting-based strategies in Parkinson's disease

Reference	Co-grafted cells	Animal model	Outcomes
Aguayo et al., 1984	Rat fVM tissue + rat sciatic nerve	6-OHDA Rat (female)	<ul style="list-style-type: none"> ▪ Monoaminergic neurons within the graft extended axons along the nerve bridges
van Horne et al., 1991	Rat fVM tissue + rat sciatic nerve	6-OHDA Rat (male)	<ul style="list-style-type: none"> ▪ Improvement in rotational behavior after co-grafting
Timmer et al., 2004	Rat fVM cells ($2-2.5 \times 10^5$) + rat SC ($2-2.5 \times 10^5$) Intrastriatal 8 wk post-lesion	6-OHDA (MFB) Rat (female)	<ul style="list-style-type: none"> ▪ Increased survival of DA cells in co-grafts
Collier et al., 1994	Monkey fVM cells + monkey saphenous nerve 1 post-lesion	MPTP Monkey (male)	<ul style="list-style-type: none"> ▪ No neuroprotective effects
Agrawal et al., 2004	Rat fVM cells (2.5×10^5) + rat OECs (2.5×10^5) Intrastriatal 5 wk post-lesion	6-OHDA Rat	<ul style="list-style-type: none"> ▪ Improvement in rotational behavior after co-grafting ▪ Increased survival of grafted DA cells ▪ Restored levels of striatal DA and DOPAC
Johansson et al., 2005	Rat fVM cells (1×10^5) + rat OECs (2×10^5) or rat astrocytes (2×10^5) Intrastriatal 4 wk post-lesion	6-OHDA (MFB) Rat (female)	<ul style="list-style-type: none"> ▪ Co-grafts with OECs enhanced DA survival and neurite elongation
Weng et al., 2017	Rat fVM cells (2×10^5) + rat OECs (2×10^5) Intrastriatal 3 wk post-lesion	6-OHDA (MFB) Rat (male)	<ul style="list-style-type: none"> ▪ Co-grafted rats exhibited better motor recovery (rotometer) and enhanced survival of grafted DA cells
Shukla et al., 2009	Rat fVM-derived NSCs (2.5×10^5) + rat OECs (2.5×10^5) Intrastriatal 4 wk post-lesion	6-OHDA (MFB) Rat (female)	<ul style="list-style-type: none"> ▪ Restitution of motor function and increased survival and reinnervation of grafted NSCs
Weng et al., 2020	Porcine fVM cells (2.5×10^5) + rat OECs (2.5×10^5) Intrastriatal 2 wk post-lesion	6-OHDA (MFB) Rat (male)	<ul style="list-style-type: none"> ▪ Better improvement in apomorphine-induced rotational behavior and higher DA survival and less immune response
Brundin et al., 1986	Rat fVM cells ($1.2-1.5 \times 10^5$) + rat fST cells ($1.5-2 \times 10^5$) or rat spinal cord cells ($1.5-2 \times 10^5$) Intrastriatal 3 wk post-lesion	6-OHDA (MFB) Rat (female)	<ul style="list-style-type: none"> ▪ Co-grafted animals showed a tendency to improve behavioral effects (rotation test) ▪ Greater area of reinnervation after co-grafts ▪ Similar number of DA cells in co-grafted rats compared to rats grafted with fVM cells alone
Yurek et al., 1990	Rat fVM cells ($6-7.5 \times 10^4$) + rat fST cells ($6-7.5 \times 10^4$) (mixed in the same cell suspension or grafted separately) Intrastriatal	6-OHDA Rat (male)	<ul style="list-style-type: none"> ▪ Larger DA cell body, arborization and improved motor behavior (rotometer) after co-grafting

Costantini et al., 1994	1 wk post-lesion Rat fVM cells (1×10^5) + rat fST cells (1×10^5) Intrastriatal	6-OHDA (LV) Rat (3 days old)	<ul style="list-style-type: none"> Co-transplants produced stronger behavioral effects and dense TH-positive patches within the grafts, without increasing DA survival
Emgard-Mattson et al., 1997	2-4 months post-lesion Rat fVM + rat fST cells (lateral and medial ganglionic eminences) ($7.9-12.7 \times 10^4$ total cells in the suspension) Intrastriatal	6-OHDA (MFB) Rat (female)	<ul style="list-style-type: none"> Co-grafts containing lateral ganglionic cells showed patches of dense TH-positive fibers No functional differences were observed after co-grafting (rotometer and staircase tests)
Sortwell et al., 1998	Rat fVM (1.8×10^5) + rat fST cells (1.8×10^5) (mixed or grafted separately) Intrastriatal 4 wk post-lesion	6-OHDA (MFB) Rat (male)	<ul style="list-style-type: none"> Only rats that received co-grafts placed separately showed significant behavioral recovery and increased DA cell survival Mixed co-grafts showed enhanced neurite branching and TH-positive rich patches
Sladek et al., 2008	Monkey fVM cells (intranigral) + monkey fST cells (at different points rostrally to the fVM graft, following the nigrostriatal pathway) 2-3 months post-lesion	MPTP Monkey	<ul style="list-style-type: none"> Striatal grafts contained dense DA axons derived from the fVM graft (target-directed outgrowth)
Lee et al., 2003	Human fVM + human fST (1 lateral ganglionic eminence) Intraputamen	PD patient (male)	<ul style="list-style-type: none"> Improved motor function and increased L-DOPA uptake
Wilby et al., 1999	Rat fVM cells (3.4×10^6 cells; intranigral) + GDNF-secreting SC line derived from rat sciatic nerve (2.5×10^5 cells; different deposits along the nigrostriatal pathway) 3 wk post-lesion	6-OHDA (MFB) Rat (female)	<ul style="list-style-type: none"> Co-grafts improved DA survival and neurite outgrowth No additional effects on motor functions
Rodriguez-Pallares et al., 2012	Rat fVM cells (4.5×10^5) + carotid body cells (4.5×10^5) Intrastriatal 6 wk post-lesion	6-OHDA (MFB) Rat (female)	<ul style="list-style-type: none"> Significantly better motor performance (cylinder test) and improved DA survival and graft-derived DA innervation in rats that received co-grafts
Ostenfeld et al., 2002	Rat fVM cells (2.5×10^5) + expanded NPCs obtained from rat fST tissue and modified to produce GDNF (4×10^5) Intrastriatal 3 wk post-lesion	6-OHDA (MFB) Rat (female)	<ul style="list-style-type: none"> Co-grafting increased DA survival No significant effects on behavioral recovery
JingZhong et al., 2005	Rat embryonic fibroblasts expressing TH + rat embryonic fibroblasts expressing GDNF (total number = 1×10^6) Intrastriatal 2 wk post-lesion	6-OHDA (MFB) Rat (female)	<ul style="list-style-type: none"> Only rats that received co-grafts showed stable and significant behavioral improvements (rotometer) and biochemical recovery (TH expression)
Deng et al., 2013	Rat fVM cells (2.5×10^5) + rat NSCs overexpressing GDNF (2.5×10^5)	6-OHDA (MFB) Rat (male)	<ul style="list-style-type: none"> Co-transplantation significantly reduced apomorphine-induced rotations

	Intrastriatal 2 wk post-lesion		<ul style="list-style-type: none"> Survival and differentiation of NSCs increased after co-grafting.
Perez-Bouza et al., 2017	Cultured rat fVM cells (equivalent to ½ VM) + encapsulated mouse C2C12 myoblasts transfected to produce GDNF Intrastriatal	6-OHDA (MFB) Rat (female)	<ul style="list-style-type: none"> Higher fiber outgrowth in co-grafted animals Similar DA survival and graft volume
Song et al., 2018	13 wk post-lesion Mouse or rat fVM-derived NSCs + mouse or rat VM- or cortex-derived astrocytes (ratio 2:1; total number = 4.5×10^5) Intrastriatal	6-OHDA (SN) Rat (female)	<ul style="list-style-type: none"> Co-grafting with VM-derived astrocytes promoted DA differentiation of NPCs and increased fiber outgrowth
Leveque et al., 2015	Porcine fVM cells (3×10^5) + rat BM-MSCs Intrastriatal 1 wk post-lesion	6-OHDA (MFB) Rat (male)	<ul style="list-style-type: none"> MSCs promoted long-term survival of DA neuroblasts and motor recovery
Rodríguez-Pallares et al., 2021	Rat fVM cells (5×10^5) + rat BM-MSCs (2.5×10^4 (low dose) or 2×10^5 (high dose)) Intrastriatal 6 wk post-lesion	6-OHDA (MFB) Rat (female)	<ul style="list-style-type: none"> Increased survival of DA nigral neurons and striatal terminals after co-grafting fVM cells and low dose of MSCs High dose of MSCs co-grafted with fVM cells decreased DA survival
Shintani et al., 2007	Rat fVM cells (8×10^4) + mouse CM-derived from BM-MSCs Intrastriatal	6-OHDA (MFB) Rat (male)	<ul style="list-style-type: none"> Increased survival of grafted DA cells
Clinical trial identifier NCT03684122	Human UC-MSC-derived NSCs ($8-12 \times 10^6$; intrathecal) + human UC-MSCs ($8-12 \times 10^6$ (intrathecal) or $4-6 \times 10^7$ (intravenous))	PD patients	<ul style="list-style-type: none"> Clinical trial ongoing

6-OHDA: 6-Hydroxydopamine; BM-MSCs: bone marrow-derived mesenchymal stromal cells; CM: conditioned medium; DA: dopamine; DOPAC: 3,4-dihydroxyphenyl acetic acid; fST: fetal striatal; fVM: fetal ventral mesencephalic; GDNF: glial cell line-derived neurotrophic factor; L-DOPA: L-3,4-dihydroxyphenylalanine; LV: lateral ventricle; MFB: medial forebrain bundle; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NPCs: neural precursor cells; NSCs: neural stem cells; OECs: olfactory ensheathing cells; PD: Parkinson's disease; SC: Schwann cells; SN: substantia nigra; TH: tyrosine hydroxylase; UC-MSCs: umbilical cord-derived mesenchymal stromal cells; wk: weeks.