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Recent advances in stem cell therapy for Parkinson's Disease: A Review

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Abstract
Parkinson's disease (PD) is a degenerative neurological condition characterized by tremor, bradykinesia, and stiffness as cardinal motor characteristics. It's linked to a long-term loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc), resulting in a severe DA shortage in the striatum, which is necessary for motor function. There is presently no cure for Parkinson's disease, and the majority of treatments aimed at reversing dopamine depletion and alleviating symptoms. The transplantation of stem cells or stem cell derived progenitors has highlighted the potential of employing cell-based therapy to replace lost cells in the sick brain, based on promising findings from early experiments. Embryonic stem cells (ESCs) are highly expandable and pluripotent cells that can differentiate into any cell type in the human body, including nervous system tissues, suggesting that they could provide a long-term treatment for Parkinson's disease and other neurological illnesses. However, because of the potential for safety and ethical difficulties involved with the use of undifferentiated ESCs in people, other sources of transplantable cells must be considered. Another method is to use external manipulation to stimulate endogenous stem cells to heal the brain.
Recent advances in stem cell research in Parkinson's disease will be discussed in this review, which will provide an overview of the various sources and strategies such as the use of different stem cell populations for cell replacement and possible modulation of endogenous stem cells, that have the potential to provide effective cell-based therapy in the future.

Keywords: Parkinson's disease, Stem cell therapy

Introduction
Parkinson's Disease (P.D), the second most common progressive neurodegenerative movement disorder characterized by selective loss of dopaminergic (DA) neurons from the pars compacta (SNc) of the substantia nigra, and the formation of Lewy bodies, although it should be noted that non-dopaminergic (non-DA-ergic) system might also be affected [1]. Clinically, the manifestation of symptoms in P.D occurs after the depletion of 70% DA-ergic neurons with both motor symptoms of bradykinesia, rigidity, tremor, followed by postural instability and non-motor symptoms including cognitive abnormalities, sleep disorders, and pain [2].
Currently, the treatment for P.D is mainly aiming to relieve the motor symptoms including the usage of L-3,4 dihydroxyphenylalanine (L-DOPA) which provides an exogenous source of DA to the striatum. Continuous or chronic usage of L-DOPA decreases the effectiveness of the drug and causes chronic side effects resulting in the manifestation of troubling motor complications such as L-DOPA induced dyskinesias and they also do not counteract the progression of the disease [3].
Therapy based on Stem cells (SC), is the emerging treatment procedure for neurodegenerative disorders including P.D. SC which are undifferentiated immature cells capable of self-renewal can differentiate into multiple specialized somatic cells including DA neurons [4]. As P.D is associated with regional loss of neurons, effective cell transplantation therapy with SC which has the potential to restore the lost neurons is an effective procedure to restore and replace the damaged tissues (neurons) with healthy ones [5]. A large variety of stem cells including embryonic stem cells (ESC), fully differentiated DA neurons from fetal brain tissue or fetal neural stem cells (fetal NSC), mesenchymal stem cells (MSCs), adult stem cells (ASC), and induced pluripotent stem cells (IPSC) reprogrammed from patient's somatic fibroblasts or blood cells are used in therapy to replacing lost DA [6]. SCT is mainly done based on two different approaches: an exogenous approach involves the introduction of a population of cells i.e. *via* transplantation into the diseased brain to replace lost cells and to support the remaining cells (cell replacement) and an endogenous approach, triggers brain repair through enhancing the differentiation, proliferation, and migration of host endogenous stem cells (endogenous regeneration) [7]. Hence, research focusing on the identification of the optimal cell type that

can offer lasting therapeutic efficacy in human PD patients is necessary and an important step in the treatment of P.D.

Sources of stem cell for P.D treatment

Several stem cell sources for the therapy of Parkinson's disease have been researched over the years, as shown in Fig. 1. Adult bone marrow-derived mesenchymal stem cells (BM-MSC) and olfactory ensheathing cells (OEC) are being used widely, however their capacity to develop into dopamine neurons are limited. To far, neural stem cells (NSCs) and dopamine neurons derived from foetal brain tissue, embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and directly induced dopamine neurons (iDA neuron) reprogrammed from autologous somatic cells have all been extensively used [8].

There are two key properties that define a stem cell: self-renewal, which is the ability to undergo numerous cycles of cell division while maintaining the undifferentiated state; and potency, its differentiation potential [9]. On this basis, stem cells can be broadly classified as being totipotent, pluripotent or multipotent. Totipotent stem cells can only be isolated from the early developing embryo (morula) and can differentiate into any cell type within the body, including extra-embryonic tissue [10]. Similarly, to totipotent stem cells, pluripotent stem cells are also capable of differentiating into any cell type so they are able to give rise to cells from any of the three major tissue lineages: the ectoderm, mesoderm and endoderm. Pluripotent stem cells are found in the blastocyst of the embryo, but unlike totipotent cells, these have already begun the differentiation process [11]. Multipotent stem cells are lineage limited, which means they can only differentiate into the cell types from which they were produced, and they can be isolated from a variety of tissues throughout the adult human body [12].

Future stem cell replacement techniques in PD will need a population of adequate DA-ergic neurons that can be easily procured, grown, and manufactured *in vitro*. DA-ergic neurons for grafting may theoretically be made in huge quantities from a variety of stem cells, including ESCs, foetal NSCs, and ASCs, all of which have various properties [13]. The use of embryonic and foetal tissue, on the other hand, is hampered by ethical concerns and limitations with tissue availability, whereas ASCs have a lower differentiation capacity. As a result, in addition to cell type-specific features, these cell types must demonstrate their potential to create functional DA-ergic neurons *in vitro* and promote therapeutic efficacy *in vivo* (i.e., in animal models of PD) before they can be used clinically in people [14].

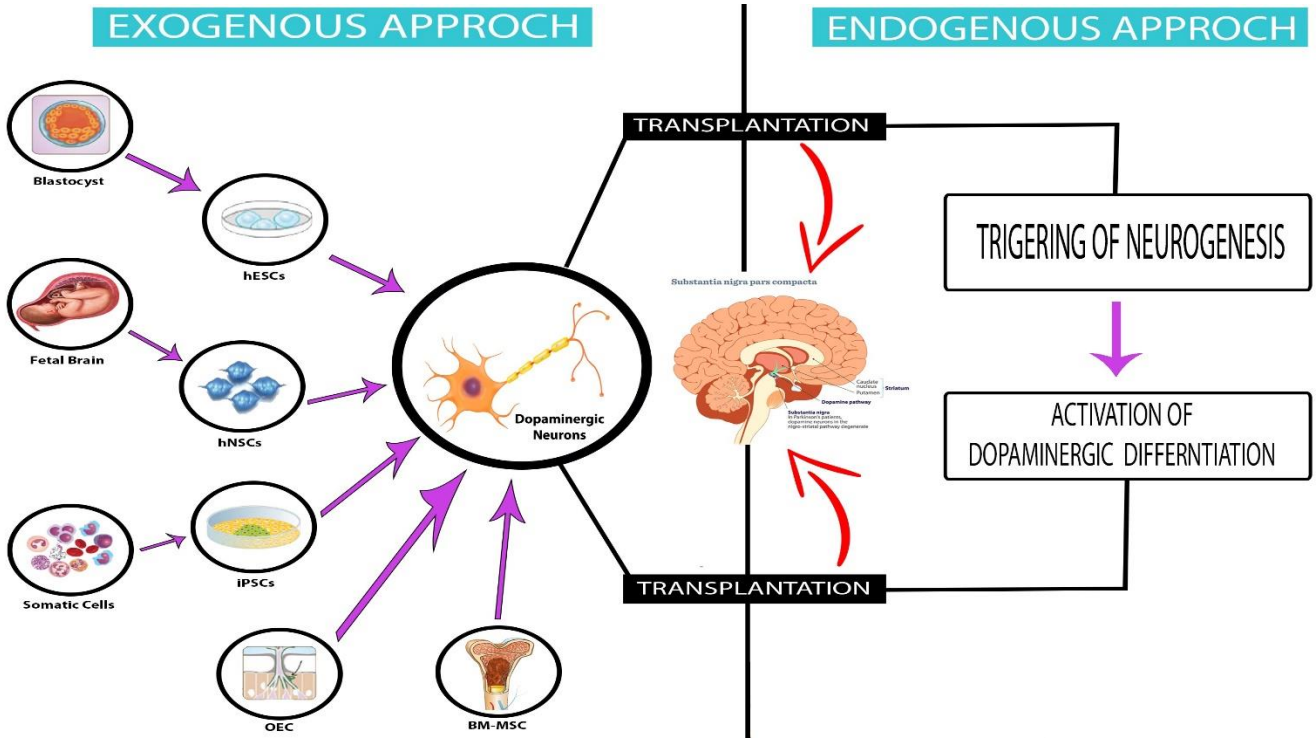

Fig 1: Schematic illustration of the different types of human stem cells sources currently available for potential application in the cell replacement therapy (CRT) for Parkinson's disease (PD)

Human embryonic stem cells (hESC)

Human embryonic stem cells (hESCs) are pluripotent, self-renewing cells extracted from the preimplantation blastocysts' inner cell mass. As a result, ESCs can be developed into any type of tissue cell, including neural stem cells (NSCs), neurons, and dopaminergic neurons (DA neurons) [15]. NSCs produced from embryonic stem cells, as well as fully developed neurons and dopamine neurons, have been found to exhibit neuroprotective properties in the treatment of Parkinson's disease. hESCs were first obtained by coculturing mouse embryonic fibroblast feeder cells with inner cell mass cells (MEFs) [16].

In the last two decades, techniques for directing hESC development into neural stem cells and neurons, namely dopamine neurons for PD, have been established. The use of particular patterning molecules that affect midbrain development *in vivo* has been found to differentiate hESCs into midbrain dopamine (DA) neurons [17, 18]. Transplanting hESC-derived neural precursor cells into PD revealed that the transplanted cells developed into DA neurons *in vivo*, indicating that a procedure for creating more DA neurons *in vitro* is needed. Different protocols are devised for producing midbrain-like DA neurons from hESC-derived neuroepithelial cells by combining the growth factors SHH and FGF8 in a

precise order. While the rate-limiting enzyme for dopamine production, tyrosine hydroxylase (TH), could be induced, the yield of TH-positive cells was very variable. Despite this, these cells have been found to survive transplanted into rodents and to cause some motor recovery [19].

In addition to the variable efficacy of differentiation techniques, it was clear that while these cells could secrete dopamine, they were not genuine nigral neurons because they lacked the expression of LMX1A and FOXA2, which were considered important markers for the nigral dopaminergic neuron phenotype [20]. The discovery that nigral dopaminergic neurons are of floor plate origin rather than neuroepithelial origin led to the invention of new differentiation techniques and the production of cells that closely resemble genuine nigral dopaminergic neurons [21]. However, as more ESC-derived brain cell grafting in animal models has been done, it has become obvious that variable yield is still an issue. An important retrospective study including over 500 grafts was conducted to evaluate the parameters that impacted graft success. The grafts with the highest concentration of TH-positive cells were found to be enriched for neural progenitor cells expressing markers identified in the caudal midbrain (e.g., CNPY1 and EN1. LMX1A and FOXA2, which were previously utilized to identify nigral phenotype, were also found in rostral midbrain progenitor cells destined for subthalamic nucleus neuron destiny [22]. This discovery explains the historically observed variation in transplant outcomes and, more crucially, suggests that dopaminergic neuron progenitors can now be generated with great purity and efficiency.

As previously noted, progress toward an ESC-derived therapy has been iterative, with significant findings in neurodevelopmental biology shifting the field's direction multiple times. However, now that we've figured out how to make dopaminergic neuron progenitors, the focus is shifting to whether these cells can be used in therapeutic settings, and a number of clinical trials for this method are about to commence.

Fetal neural stem cells (fetal NSC)

NSCs are stem-like progenitor cells extracted from foetal brains or particular areas of adult brains. The subgranular zone (SGZ) of the hippocampus's dentate gyrus and the subventricular zone (SVZ) of the lateral ventricles are two restricted locations that produce NSCs in adult tissue, and these two regions confer neurogenesis in the adult brain. NSCs are multipotent stem cells that mimic the neural lineage's developmental limitation [23]. As a result, neurons, astrocytes, and oligodendrocytes could be formed from these cells. The likelihood of tumour formation is reduced as a result of this precise lineage restriction, and NSCs are more easily steered toward neuronal differentiation [22]. However, these cells are difficult to isolate in high numbers, and maintaining or expanding them *in vitro* for lengthy periods of time is similarly difficult. As a result, NSC transplantation applications are currently limited [24].

Mesenchymal stem cells (MSCs)

Mesenchymal stem cells are multipotent, non-hematopoietic cells that are extracted from the stromal portion of adult bone marrow [14]. MSCs can be derived from a range of non-marrow tissues, including the placenta, muscle, skin, dental pulp, adipose tissue, umbilical cord, and amniotic fluid, in addition to bone marrow [25]. MSCs may be exempt from ethical problems because they can be extracted from adult tissues. Immunomodulation is another unique property of MSCs, which may allow the cells to evade the host's immune system's monitoring or lower the immunological response of the host. This trait would be a major problem if it were to be used in transplantation [26, 27, 28].

Induced pluripotent stem cells (iPSC)

The procedure for creating iPSCs was initially disclosed in 2007, opening up a new route for the development of a stem cell-based treatment for Parkinson's disease (and a variety of other diseases. The expression of a number of transcription factors that can promote pluripotency (e.g., c-myc, klf-4, sox2, and oct4) allows an adult somatic cell (such as a dermal fibroblast) to be reprogrammed into a stem cell [29]. The iPSCs created in this method can be differentiated into dopaminergic neurons using protocols similar to those used with ESCs, potentially paving the way for a cell-based treatment for Parkinson's disease [30]. The advantage of iPSC-derived grafts over ESC-derived grafts is that autologous grafts might be generated by employing a patient's own fibroblasts to manufacture a neural grafting product, eliminating the need for immunosuppression that ESC-derived grafts would require [31].

In monkeys with MPTP-induced nigral toxicity, iPSC-derived neural grafts have shown encouraging effects. The implanted neural progenitors eventually expanded neurites into the striatum, formed no tumours, and enhanced motor function after two years. Clinical studies in people, like the ESC-approach, are on the horizon and will commence in the next years [32].

Directly induced dopamine neurons (iDA neurons)

Though iPSCs have significant potential for cell-based therapy for Parkinson's disease, the time-consuming procedures for generating, characterisation, and differentiation into DA neurons force researchers to look for alternate ways to obtain DA neurons [33]. Furthermore, undifferentiated cells in human iPSC-derived neural stem cells or dopamine neurons may cause tumour growth and limit their clinical applicability. The ability to generate iDA cells directly from somatic cells could have major ramifications for understanding essential events in brain development *in vitro* disease models and cell replacement therapies. Another strategy is to convert PD patients' fibroblasts into DA neurons directly (iDA neurons) [34]. Recent studies has shown that directly reprogramming fibroblasts with diverse combinations of transcription factors Mash1 (Ascl1), Nurr1 (Nr4a2), Lmx1a, Ngn2, Sox2, and Pitx3 can result in the generation of DA neurons.

Most direct reprogramming approaches use lentiviral vectors to express genes involved in DA neuron growth, these transgenes may integrate into the iDA neurons' genome and cause mutagenesis [35]. Furthermore, as we highlighted in iPSCs, fibroblasts from PD patients may harbour genetic alterations in genes including SNCA, Parkin, LRRK2, and GBA. These difficulties may raise questions about the safety of using directly altered DA neurons in therapeutic settings. However, advances in science will eventually overcome these obstacles, allowing these cells to be tested in clinical trials for Parkinson's disease [36].

Future aspects and for cell-based therapy of PD

Although there are a number of hurdles associated with stem

cell-based treatments for Parkinson's disease, it appears likely that these treatments will reach the clinic in the near to medium term. While the creation of improved goods has to be long and incremental, the field is now raising concerns about how these therapies may be scaled and delivered—a sign of how far these approaches have progressed [29].

As previously stated, stem cell treatments are mostly used to treat the motor symptoms of Parkinson's disease [37]. They will not have any disease-modifying effects, and they will not cure the major non-motor symptoms, which can be extremely debilitating in some people. While these approaches may represent one arm of the future of PD treatment, they will very certainly be paired with other innovative alpha-synuclein pathology-targeting therapeutics (e.g., immunotherapies and re-purposed drugs) [38]. It may be possible to deploy stem cell-based regenerative therapies to restore function to dopaminergic neurons that have already died, while innovative disease-modifying medications could be used to prevent neuronal loss from continuing [39].

Conclusion

Cell replacement therapy holds promise as a treatment for Parkinson's disease and other neurodegenerative diseases. The use of any derived cell source - foetal NSCs, ESCs, iPSCs, and iDA neurons – raises ethical, logistical, and safety considerations. Scientific research, on the other hand, is making significant progress in the generation and characterization of iPSC-derived cells for Parkinson's disease. Injections of iPSCs and their derivatives into animal models have showed promise in the treatment of illnesses such as Parkinson's disease; nevertheless, iPSCs have not been used in clinical studies for Parkinson's disease. There are some drawbacks and restrictions of using iPSCs. In such treatments, an appropriate therapeutic progenitor or mature cell type can be selected and transplanted; in the case of PD, the options include iPSC-derived NSCs and iPSC-derived DA neurons, of course. Theoretically, these two should behave similarly to their non-iPSC derived counterparts; but, due of the difficulties outlined above, the unique iPSC ancestry of such cells can occasionally produce its own set of issues.

Pre-clinical viability studies may also be required to determine the treatment's scope. At least until iPSCs are better understood and effective and safe procedures for reprogramming and gene correction are established, iPSCs will not be pushed to clinical trials. The rate of progress will undoubtedly continue to accelerate in the next years, and it is therefore very feasible that we will see the transition from dish to clinic during our lifetime.

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