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Stem cells transplantation as a treatment for Parkinson's disease

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Highlights

- **Parkinson's disease is a neurodegenerative disorder, which is suboptimally treated in the majority of patients.**
- **Current treatment strategies are directed toward treating symptoms and improving daily functional activity for the patients, but not radically solve the issue in those patients.**
- **Stem cells have shown a promising results in restoring the lost dopaminergic neurons in many preclinical and few clinical studies.**
- **Different types of stem cells, and multiple differentiation protocols have been proposed in order to choose the type with least possible complication.**

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ABSTRACT

Parkinson's disease is a progressive disorder of movement that occurs mainly in the elderly. It is the second most common neurodegenerative disease after Alzheimer's disease. Current pharmacological therapies focus on the substitution of dopamine, although this gives therapeutic benefits, its continued use is associated with reduced clinical benefits and it may be associated with a major motor complication known as dyskinesia. Due to their ability to transdifferentiate to dopamine producing cells, stem cell transplantation could be a long-term solution for the disease. Although many preclinical studies showed that different types of stem cells could improve the motor behavior in animal models of Parkinson's disease, there seem to be some technical challenges in the way of testing these cells on patients with the disease. This review highlights the recent advances in the use of three different types of stem cells; embryonic stem cells, neural stem cells, and other stem precursor cells. It particularly focuses on the complications that may arise from using each type of cell, summarizing some of the preclinical studies. Recent clinical studies using different types of cells were also reviewed.

1. Introduction:

Parkinson's disease (PD) is a progressive disorder of movement that occurs mainly in the elderly. It is the second most common neurodegenerative disease after Alzheimer's disease (Vila *et al.*, 2004). It is characterized by the selective loss of dopaminergic neurons in the substantia nigra pars compacta and the degeneration of nerve terminals in the striatum (Hornykiewicz, 1973). To compensate for the progressive loss of dopamine in Parkinson's disease, current pharmacological therapies focus on substitution of dopamine, although this gives therapeutic benefits, its continued use is associated with reduced clinical benefits and it may be also associated with a major motor complication known as dyskinesia. Similarly, deep brain stimulation (DBS) of the pallidum or the subthalamic nucleus (STN) is effective, but as the disease progress the treatment efficacy tend to decline, and the long-term benefits of pallidal or STN stimulation beyond 4-5 years remain to be established (Kleiner-Fisman *et al.*, 2003; Liang *et al.*, 2006). As an alternative therapeutic strategy to pharmacological substitution or deep brain stimulation, cell based therapy has been developed where transplantation strategies have been designed to replace lost dopamine neurons. Preliminary studies have concentrated on transplanting readily available cell sources, such as catecholaminergic adrenal medullary tissue into the striatum but limited clinical benefits were observed and the survival of grafted cells were poor (Waters *et al.*, 1990). Then, a major advancement came in the field with the use of foetal ventral mesencephalic tissue, but the development of this treatment has been hindered by technical and ethical issues surrounding the need to use a large number of aborted foetuses to treat each patient. Hence it has begged the demand for alternative tissue sources. In this regard, stem cells may constitute one such source. Thus, much work has focused on the development of mesencephalic dopamine neurons from stem cells.

What are stem cells and what makes them suitable for cell replacement therapy? They are undifferentiated, unspecialized cells, which are able to self-renew and can give rise to various specialised functional cell types. Thus, the unique properties of these cells allow their differentiation into dopaminergic neurons. This review will discuss common sources of stem cells used for the treatment of Parkinson's disease, summarizing many of the published preclinical and clinical studies performed in this field. Furthermore, the main obstacles in the way of developing and using each kind of these cells as a treatment option would be highlighted.

2. Types of stem cells used for the treatment of Parkinson's disease:

- Embryonic stem cells (ESCs)
- Neural stem cells (NSCs): from embryo, fetus or adult.
- Other stems/precursor cells: e.g. bone marrow, umbilical cord, skin (Lindvall *et al.*, 2004).

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2.1 Embryonic stem cells (ESCs)

They are pluripotent cells that provide an unlimited source of material and can give rise to all cells of the body except the placenta. Although these properties make them suitable candidates for cell replacement therapy, studies have shown that transplantation of undifferentiated mouse ESCs into the striatum of Parkinson's disease rat model significantly increased the risk of teratoma formation (Bjorklund *et al.*, 2002). This poses the question of how can ESCs be restricted to produce useful cells without overgrowing. Several *in vitro* methods have been developed to differentiate mouse ESCs into midbrain dopamine neurons whilst removing the undifferentiated cells that could potentially grow into teratoma. They included methods that involved culturing cells with extrinsic signaling factors, like a signal sonic hedgehog (Shh), and fibroblast growth factor 8 (FGF8), which are known to influence the local environment, in which mesencephalic dopamine neurons are generated during embryogenesis. These studies resulted in the increase of functional dopaminergic neurons *in vitro* (Lee *et al.*, 2000), and *in vivo* (Kim *et al.*, 2002) and no teratoma observed at the time of analysis. However, these signaling factors could also underlie the regional specification of other cell types. Consequently, if cultures include other neurons like serotonergic neurons, these contaminating neuronal types may cause unwanted side effects after grafting. This emphasizes the importance of methods that can produce highly enriched cultures of dopamine neurons.

One such study shows that the forced expression of the transcription factor Lmx1a in ESCs selectively promotes the production of functional dopamine neurons, suggesting that the expression of Lmx1a eliminates the production of harmful 5-HT neurons. The experiment used a magnetic sorting strategy to eliminate contaminating cells that would potentially lead to overgrowth, resulting in no evidence for teratoma formation at the time of analysis (Firling *et al.*, 2009). Another independent ESCs study suppressed the EGF-CFC protein Cripto, which expresses in multiple cancer cells. This study demonstrated that in vitro differentiation of Cripto$^{-/-}$ESCs resulted in increasing dopamine cell differentiation and after transplantation into a rat model of Parkinson's disease, resulted in behavioral and anatomical recovery without tumor formation (Parish *et al.*, 2005). Although enrichment of dopaminergic neurons is important for cell replacement therapy, the survival of these neurons post-graft is critical for their long-term function. One study generated mouse ESCs overexpressing the antiapoptotic factor Bcl-XL, which resulted in the differentiation of more dopaminergic and serotonergic neurons compared with wild type control ESCs. Upon transplantation, those neurons exhibited more extensive fiber outgrowth and more behavioral asymmetry improvement than wild type (Shim *et al.*, 2004). Moreover, the dopamine neurons derived from Bcl-XL overexpressing ESCs were less susceptible to dopamine neuron neurotoxin, suggesting that Bcl-XL might have a neuroprotective effect.

Since this study aims to treat Parkinson's disease in human patients, similar differentiation protocols have more recently been developed to generate functional dopamine neurons from human embryonic stem cells (hESCs) (Fig. 1). Although dopamine neurons can be derived from hESCs *in vitro,* there is some evidence that these neurons do not survive *in vivo* after transplantation. Park *et al*. used an *in vitro* differentiation protocol to generate dopamine neurons from hESCs co-cultured with stromal cells and specifically expressed transcription factors related to midbrain development. Although the hESCs derived dopamine neurons functioned *in vitro*, and released dopamine in response to potassium chloride-induced depolarization, when these derived dopamine neurons were transplanted into the striatum of hemiparkinsonian's rats, they failed to improve behavioral deficits. Further immunohistochemical analysis showed the hESCs derived cells survived in the grafts, but none displayed typical dopamine neuronal markers (Park *et al.*, 2005).

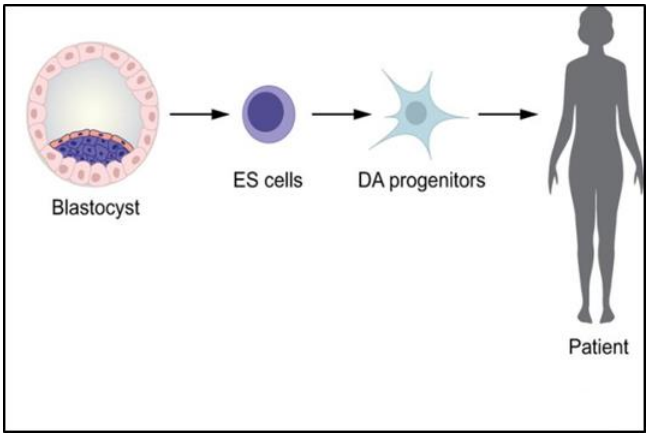

Fig. 1. Schematic representation of in vitro differentiation of hESCs to dopaminergic neurons, and transplantation of mature dopaminergic neurons.

On the other hand, several experiments showing that hESCs derived dopamine neurons can survive *in vivo* after transplantation. For example, Sonntag *et.al.* generated dopaminergic neurons from hESCs utilising a differentiating protocol that involved the combining use of a bone morphogenic protein antagonist noggin and culturing with stromal feeder cells. The first induces neuroectodermal cell development, and the second induces the production of dopaminergic neurons. He demonstrated that the derived cells could function *in vivo* animal models. However, this differentiation protocol did not entirely exclude the development of teratoma formation in nearby regions (Sonntag *et al.*, 2007). In addition, an earlier study that generated neural progenitors *in vitro* from hESCs using the bone morphogenic protein antagonist noggin showed that the derived neural progenitors from hESCs upon transplantation into a rat model of Parkinson's disease survived. This specific transplantation study also caused partial behavior improvement and no teratoma (Ben-Hur *et al.*, 2004). As demonstrated by the studies described above, it is unclear whether hESCs can survive *in vivo* or not. This difference may be contributed to alternate technical methods used in the preparation procedure or transplantation procedure. Also, it might be that cells derived from hESCs are unlike mESCs derived dopaminergic cells that showed to survive and function, although further studies will be required to clarify this. Another factor needs to be clarified is whether the *in vitro* differentiation prior to transplantation will improve or worsen the *in vivo* survival of hESCs. To determine whether or not the pre-differentiation of hESCs *in vitro* improves *in vivo* survival and prevents teratoma formation. A study (Brederlau *et al.*, 2006) used PA6 stomal cell line for *in vitro* culture found that *in vitro* pre-differentiation decreased the number of viable cells present in the grafts. In contrast, teratoma formation was found to decrease with differentiation time suggesting that the pre-differentiation stage *in vitro* could reduce unwanted cells and reduces the risk of teratoma formation. Further work is needed to find the best protocol for producing a high yield of dopamine neurons from ESCs that can survive, integrate and function *in vivo* without overgrowth. Some researchers moved away from ESCs that can grow to all cell types and undifferentiated cells to NSCs that restrictively grow only into nerve cells.

2.2 Neural stem cells (NSCs)

They are multipotent cells, which can self-renew but remain more restricted than ESCs and are often defined by the organ in which they reside. These cells can differentiate into many types of nerve cells (Fig. 2). It has been shown that NSCs not only exist in the developing brain, but also the adult brain (Palmer *et al.*, 2001). There is evidence for neurogenesis in the adult mammalian substantia nigra for the type of dopaminergic neurons that are lost in Parkinson's disease, and the rate of neurogenesis is found to increase after a lesion (Zhao *et al.*, 2003). Theoretically, Parkinson's disease may be caused by a decrease in neurogenesis rather than

cell death. It can also be speculated that the endogenous NSCs neurogenesis may be slow in the adult brain, so they cannot cope with the loss of dopaminergic neurons in Parkinson's disease. Hence trying transplantation of foreign NSCs is needed.

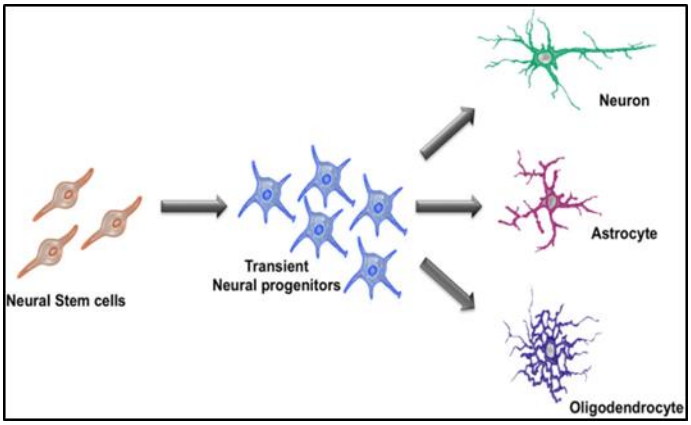

Fig. 2. Schematic representation of neural stem cell derivatives.

As mentioned previously, the production of dopaminergic neurons from NSCs is considered to be more limited than ESCs. Therefore, protocols have been developed to increase the efficiency of the generation of dopaminergic neurons from NSCs. Liste *et al.* overexpressed the antiapoptotic factor Bcl-XL in human NSCs and showed that Bcl-XL overexpression increased the dopaminergic differentiation of human NSCs both *in vitro* and *in vivo.* Importantly, the transplanted graft of Bcl-XL-overexpressing human NSCs generated surviving dopaminergic neurons in the striatum of a rat model. Implying that Bcl-XL not only increases differentiation but also prolongs the survival of derived dopaminergic neurons and the effect seems to be more prominent than that previously mentioned in mouse ESCs by Shim *et al.* (2004) and Liste *et al.* (2004). When neurturin (NTN), a member of the glial cell line-derived neurotrophic factor (GDNF) family was overexpressed in NSCs, its presence provided dopaminergic neuroprotection effects after transplantation into the striatum of rat models (Liu *et al.*, 2007a). In another set of related experiments, Liu *et al.* (2007b) demonstrated that neurturin overexpressing NSCs exerts a neurogenerative effect upon transplantation into the striatum of rat models. A further study used the transplantation of human NSCs cloned by v-myc gene transfer showed that these NSCs have neuroprotective effects against dopaminergic depletion in vitro and in vivo. This protective effect is obtained by suppressing apoptosis through up-regulating the antiapoptotic protein Bcl-2. These transplanted NSCs significantly ameliorated parkinsonian behavioral symptoms (Yasuhara *et al.*, 2006). A further study has demonstrated that treatment by the neurotrophic factor cerebrolysin (CBL) as an adjuvant to neural stem cell transplantation can stimulate the survival of the grafted cells in the transgenic mouse model of Parkinson's disease (Rockenstein *et al.*, 2015). It is clear from the above studies that transplantation of NSCs beside their functional replacement role, they have neuroprotective effects. Adult NSCs may also allow autologous transplantation that would avoid the use of immunosuppressants to prevent immune rejection due to immunological incompatibility between the donor and receiver. However, their location within the adult central nervous system makes them impractical for surgical removal and autologous transplantations. Researchers have yet to find readily accessible sources to avoid immunity related complications.

2.3 Other stems/precursor cells

They are harvested from tissues like an umbilical cord, bone marrow, skin, and olfactory mucosa. They are considered as readily accessible sources for autologous grafting. Stem cells derived from the human umbilical cord matrix Wharton's Jelly, are called umbilical cord matrix stem cells (UCMS cells), and they are a source for mesenchymal stem cells (MSCs). A pilot study using undifferentiated human UCMS cells transplanted into hemiparkinsonian rats that were not immunosuppressed resulted in amelioration of rotational behavior induced by apomorphine. Additionally, when UCMS cells were transplanted into healthy normal rats, they did not cause any tumors, rotational behavior, and no immune rejection response was observed (Weiss *et al.*, 2006). This suggests that the umbilical cord matrix is a source for primitive mesenchymal stem cells, and it can be therapeutically useful in the treatment of Parkinson's disease.

Mesenchymal stem cells (MSCs) from bone marrow stromal cells could be induced to produce dopaminergic neurons *in vitro*. Transplantation of the induced cells showed improvement of rotational behavior induced by apomorphine in Parkinson's disease rat models (Dezawa *et al.*, 2004). These findings indicate that the transdifferentiated MSCs from bone marrow could be a source for cell replacement therapy for the disease. An independent but related study has compared the MSCs isolated from Parkinson's patient's bone marrow with those derived from normal adult bone marrow. Findings indicated that the characterization of both is similar. Moreover, the MSCs derived from Parkinson's disease can successfully be differentiated into dopamine neurons. Also, Parkinson's disease derived MSCs could inhibit T lymphocyte proliferation induced by mitogen. These findings suggest that MSCs from Parkinson's disease patient bone marrow can be an autologous source for stem cell replacement therapy in Parkinson's disease (Zhang *et al.*, 2008).

It is also possible for multipotent stem cells derived from adult mammalian skin dermis to differentiate into neural cells (Toma *et al.*, 2001). In the year 2007, a study described the creation of induced pluripotent stem cells (iPSCs) from adult human dermal fibroblasts by retroviral transduction of four defined transcription factors (Fig. 3). It has been shown that human induced pluripotent stem cells were comparable to hESCs in morphology, proliferation, surface antigens, gene expression, and teratoma formation (Takahashi *et al.*, 2007). A year later, another study showed that reprogrammed fibroblast into pluripotent stem cells (induced stem cells) that was achieved through retroviral transduction of four transcription factors could integrate and decrease symptoms of Parkinson's disease in a rat model (Wernig *et al.*, 2008).

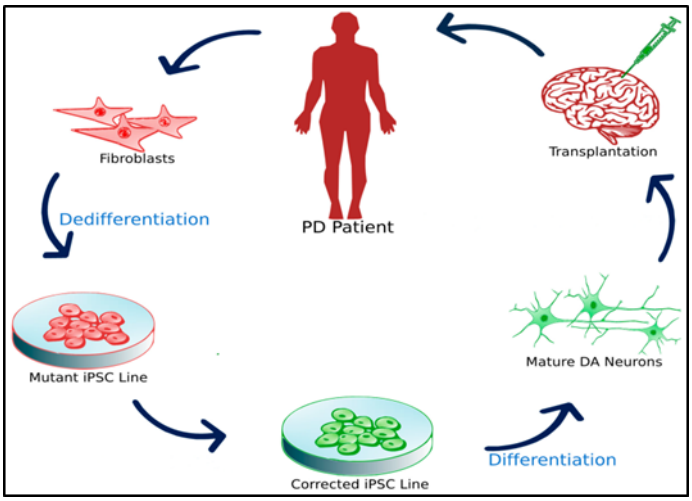

Fig. 3. Fibroblast reprogramming to produce iPSCs that can differentiate to dopaminergic neurons in vitro modified from (Hallett *et al.*, 2015).

Human MSCs also have been shown to have a neuroprotective effect. One study showed that the human MSCs derived from an adult bone marrow reduced dopaminergic neuron loss induced by a proteasome inhibitor (MG-132) *in vitro* and *in vivo* and significantly increased the survival of dopaminergic neurons by 50% (Park *et al.*, 2008). Lu et al. have tried to deliver the gene of the rate-limiting enzyme, tyrosine hydroxylase (TH) that is involved in the biosynthesis of dopamine, by using MSCs as a vehicle. These TH-engineered MSCs significantly decreased rotational behavior upon

transplanting into the striatum of Parkinson's disease rat, supporting the role of MSCs in gene therapy for Parkinson's disease. However, the dose and timing expression of gene expression in the brain was hard to control and the identity of these cells that express TH^+ in the brain is not clear (Lu *et al.*, 2005).

Human olfactory neurosphere-derived cells were shown to improve amphetamine-induced rotational behavior in hemiparkinsonian's rats. The same effect was seen with olfactory biopsies obtained from healthy humans and parkinsonian's patients which differentiated *in vitro* before transplantation (Murrell *et al.*, 2008) implying that olfactory neuroepithelium which some researcher believe that it is NSCs, can be a source for autologous stem cell therapy for Parkinson's disease.

Although autologous transplantation provides an immunological advantage, it might be the patient's cells carry information that might increase the pathogenetic process underlying the disease or might be more vulnerable to the degeneration by the disease. Finally, stem cells are also useful in drug discovery, and understanding the cause and the mechanism underlying neuronal cell death in Parkinson's disease through the development of disease models, reviewed by (Jakel *et al.*, 2004).

3. Clinical studies:

Despite the massive preclinical research about the use of different types of stem cells in the treatment of Parkinson's disease, little clinical studies were performed. Since protocols of studies may raise ethical concerns for volunteer patients and financial concerns for producing companies, the involvement of the small number of patients could yield concrete evidence about treatment success. Some of the recent clinical studies where different types of stem cells were used are summarized in Table 1 below.

Table 1

Summary of the available clinical trials about the use of different types of stem cells to treat Parkinson's disease.

Intervention	Number of participants	Follow up period	Results	Reference
Allogenic MSCs transplantation.	8	12 months	Significant improvement in the score of motor behaviour (UPDRS).	Venkataramana *et al.* (2012).
Autologous MSCs transplantation.	7	36 months	3 out of 7 patients showed improvement in motor behaviour.	Venkataramana *et al.* (2010)
Trial of ESCs on Chinese patients	No published information	-	The trial is running.	Cyranoski D. (2017).
A trial to explore the safety and efficacy of iPSCs in Parkinson's disease treatment	No published information	-	Trial has not ended.	Kyoto University (2018)
Use of autologous NSCs graft	10	Estimated to be 2 years.	Trial has not finished yet	(NCT03815071)

4. Conclusion:

Based on the current, available experiments stem cells have shown to induce behavioral improvement in Parkinsonan animal models after transplantation not only as dopamine cell replacement but also by having neuroprotective effects. However, most studies have not demonstrated whether stem cells can substantially innervate the striatum and, there is no enough clinical evidence to support treatment success. For stem cells to work, they have to exert competitive advantages over the other available therapies and improve drug-resistant symptoms. Moreover, the problems associated with stem cells have to be solved. Longer time studies are needed since there is evidence suggesting that ESCs are more prone to produce teratomas when implanted into the same species from which they were derived. This suggests that even if no teratoma was seen in the animal model, it does not exclude its possible occurrence in humans. Moreover, it has to be considered if the graft can be affected by Parkinson's degeneration process.

It can be believed that stem cell therapy needs longer time studies to prove its safety and efficacy before moving into clinical trials in Parkinson's disease patients. In addition, more studies should concentrate on the neuroprotective effects of stem cells in the treatment of the disease, because despite all the research no clinically approved neuroprotective drug has been developed yet. This is partly because the exact mechanism or mechanisms that lead to neuronal cell death is not known. Thus, if stem cell transplants work to produce a neuroprotective effect and can delay the progress of the disease, it will make a great significant improvement in Parkinson's disease treatments.

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