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Cell Therapy for Parkinson’s Disease: Failure or Success?

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1. Introduction

The mature central nervous system (CNS) is probably the most complex structure known in nature. This fact and the irreversibility of most forms of clinical brain damage are the basis for the long-held belief that the adult brain cannot restore itself and cannot be repaired. Parkinson’s disease (PD) is a chronic and progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNPC) with a concomitant loss of the catecholamine called dopamine (DA), the neurotransmitter released at the axon terminals of the SNPC neurons that project to the striatum (caudate nucleus and putamen) (Fig. 1).

Historically, the therapy for PD is aimed at re installing proper stimulation of the dopamine receptors in striatum. The dramatic breakthrough was the introduction of L-DOPA treatment in 1969 (Cotzias et al., 1969). L-DOPA, the precursor of dopamine, passes the blood-brain barrier (BBB) and is converted to dopamine, which becomes available for dopamine receptors in striatum, thereby improving the balance between excitatory and inhibitory influences in this brain region. Together with L-DOPA treatment, dopamine reuptake inhibitor, dopaminergic agonists and muscarinic antagonists also have a clinical effect. Despite this pharmacologic advance in treatment, there remains no cure for PD. Because PD is a neurodegenerative process and long term therapy is necessary, development of severe side effects such as dyskinesias (movement disorder), limits the usefulness of L-DOPA therapy over time and progressively becomes less effective; consequently, patients become more troubled by freezing or akinesias. In addition, L-DOPA will not only reach the striatum, but the entire CNS as well as the rest of the body, where it can develop unwanted side effects.

Additionally, surgical treatment is being used to treat people with advanced PD for whom drug therapy is no longer sufficient. The more frequently employed techniques are thalamotomy, lesion of the internal globus pallidus or subthalamic nucleus and chronic implantation of electrodes for deep brain stimulation, amongst others. Even though there is a clinical recovery in PD patients after surgical therapy, as seen with pharmacological therapy, the progression of the disease cannot be avoided. Hence, the basic principle of
neural transplantation for PD is to provide DA from the graft in a stable fashion directly into the striatum where the intrinsic dopaminergic system has been degenerated (Fig.1). This procedure attracted the attention of the entire scientific community over the past decades. In this chapter we will describe the experimental work and clinical trials that provided the basis for the development of cell therapy in PD. Afterward, this chapter will depict the suitability and the therapeutic potential of stem cells from different origin that could be employed for regeneration therapy in human clinical trials. Finally, this chapter will discuss the current strategies for the assessment of tissue integration after grafting and our proposal of an alternative method for evaluating the effectiveness of the transplants in rodent models of PD.

Fig. 1. Transplant therapy in a rodent model of PD. Grafted cells are located in the denervated striatum of the 6-hydroxydopamine (6-OHDA) lesioned rat.

2. The experimental approach in animal models of PD: the foundation of transplant therapy

Even though PD is mostly idiopathic, it is well known that loss of a specific and highly specialized neuronal subpopulation underlie the process of the disease. The optimal tactic to evaluate the benefits and/or drawbacks of different treatments, devoid of using human

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patients, is to develop an animal model that resembles the pathology observed in PD. To this point, animal models of PD are the best method to evaluate the success or failure of transplant therapy.

Section 2 depicts the evolution of transplant research done in animal models. A summary of the trials can be found in Table 1.

2.1 Animal models of PD
The most commonly used PD model is the 6-OHDA unilateral lesioned rat. 6-OHDA is a specific neurotoxin for catecholaminergic neurons; uptake of 6-OHDA by these neurons is performed in a similar fashion to that for intrinsic catecholamines (Sachs & Jonsson, 1975). Because 6-OHDA is not able to cross the BBB, unilateral direct injection into the substantia nigra or into the medial forebrain bundle is sufficient to destroy >95% of the midbrain dopaminergic neurons. Unilateral lesioned rats will rotate contralaterally in response to DA agonists such as apomorphine, which is the result of the supersensitivity of striatal DA receptors in the lesioned side. On the opposite, unilateral lesioned rats will rotate ipsilaterally to the lesion in response to amphetamine. Circling behavior can be analyzed by gross visual observation, video recording or rotometer.

Another neurotoxin commonly employed for the development of animal models of PD is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This toxin was discovered in young drug addicts who used illicit drugs contaminated with meperidine, causing symptoms very similar to idiopathic PD (Langston et al., 1983). Sensitivity to MPTP varies considerably among animal species. Non-human primates are most sensitive in a manner similar to humans; mice, cats, and dogs are rather sensitive, whereas rats are resistant to MPTP (Kopin & Markey, 1988).

2.2 Fetal substantia nigra transplants
With the extensive variety of animal models of PD, experimental transplant therapy for PD swiftly began in the late 70’s; fetal substantia nigra was the initial choice for transplants, since fetal tissue was considered to have lower possibility of rejection from the host’s immune system than adult cells.

The firsts fetal substantia nigra experimental transplant performed in a PD model, reported a reduction of the motor abnormalities in a unilaterally DA-denervated rat (Björklund & Stenevi, 1979; Perlow et al., 1979). Since then, the technique has been improved in several ways and established that DA-containing grafts can alleviate some of the symptoms in different experimental models of PD, including mice and rats (Aguayo et al., 1984; Annett et al., 1994; Brundin et al., 1988; Di Porzio & Zuddas, 1992; Dunnett et al., 1981; Hefti et al., 1985; Herman et al., 1991; Zhu et al., 1992).

Once the first experiments using fetal substantia nigra grafts in rodents were concluded and giving preliminary and promising results, the next rational step was to extrapolate these experiments to a higher member of the evolutionary chain, the non-human primates. Morihisa et al., were the first ones to explore the potential of this therapeutic approach, transplanting fetal substantia nigra into the denervated caudate nucleus of the rhesus monkey with unilateral lesion of the striatum secondary to 6-OHDA direct administration. For their surprise, under the specific conditions of their experiment, fetal substantia nigra graft did not survive in either of two animals tested. The complete loss of the transplanted dopaminergic graft could be due to an immunologic incompatibility; which placed the notion that the brain is an immunologically privileged site under inquiry and questioned the
idea that rejection processes would be less severe inside the brain. In order to circumvent graft rejection, they employed grafts from autolog adrenal medulla tissue; of particular interest is that adrenal medulla tissue from four animals tested did survive (Morihisa et al., 1984). Even though they did not report improvement after adrenal medullary chromaffin transplant, they were the pioneers in the usage of autologous transplants for PD treatment, one that took notoriety in the years to come.

2.3 Sympathetic neuron transplants
The potential therapeutic value of autologous transplants was also explored using sympathetic neurons for cell transplant in PD. Sympathetic ganglion cells mainly release noradrenaline whereas a minute amount of DA is also synthesized and secreted by a small subpopulation of the ganglion cells (Lyon et al., 1987). With this taken into consideration, autologous sympathetic neurons derived from the superior cervical ganglion were grafted in animal models of PD, monkey and rat. Tissue pieces of the sympathetic ganglion were grown in serum-free medium supplemented with nerve growth factor; short neurites were found to emanate from sympathetic ganglion tissue after 6 days in culture. These studies have revealed that sympathetic neuron autografts placed in the DA-denervated striatum survive and improve motor deficits such as drug induced circling behavior and hypokineti 
cdisorders, seen in rodent and non-human primate models of PD (Itakura et al., 1988; Nakao et al., 1995).

2.4 Human retinal pigment epithelial (hRPE) cells transplant
With the arrival of new and more advanced cell culture procedures, innovative sources were available for the acquisition of alternative DA-producing cells. Amongst them, the hRPE cell was one of the most promising ones; the study of hRPE cells demonstrated that these cells express vesicular monoamine transporter and the D1 receptor. Moreover, hRPE cells secrete L-DOPA and small quantities of dopamine. This alternative graft was transplanted in a rat and monkey model of PD with significant improvement of circling behavior and motor task, respectively. Nevertheless, a thorough depiction of dopaminergic transplanted cell survival and function was not provided (Doudet et al., 2004; Subramanian et al., 2002).

2.5 The dim truth about experimental transplant in animal models of PD
Regardless of the improvement reported in these animal models of PD after transplant, several factors were not taken into account in order to make an adequate evaluation of the enhancement produced by the graft. Even though there is evidence of a decrease in circling behavior, the survival of DA-producing transplanted neurons is not fully described in many of the experiments, and for that reason, a correlation between neuronal graft survival and behavior improvement cannot be supported. With the purpose of making an un-doubtful conclusion that a functional recovery is clearly undergoing, a clear correlation between the extent and location of dopaminergic re-innervation within the striatum and recovery of dopaminergic function should be done. The remission of circling behavior may relate to the fact that DA receptors on the denervated side are supersensitive and/or that striatum is normally supplied with a far greater innervation than is needed for minimal maintenance of function. It is also possible that a low number of neurons and terminals may increase their synthesis and turnover of DA to help compensate for the deficit.
<table>
<thead>
<tr>
<th>Cell Source</th>
<th>Donor</th>
<th>Recipient</th>
<th>Result</th>
<th>Time period</th>
<th>TH neuron count (after transplant)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal 19 days Nigra</td>
<td>Rat</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Decrease in 33% of ipsilateral rotation (amphetamine)</td>
<td>3.5 months</td>
<td>300 neurons</td>
<td>Björklund &amp; Stenevi, 1979</td>
</tr>
<tr>
<td>Fetal Nigra</td>
<td>Rat</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Decrease in 70% of contralateral rotation (apomorphine)</td>
<td>4 weeks</td>
<td>NA</td>
<td>Perlow et al., 1979</td>
</tr>
<tr>
<td>Fetal 19 days Nigra</td>
<td>Rat</td>
<td>6-OHDA bilateral lesion Rat</td>
<td>Decrease in 50% of spontaneous akinesia (amphetamine)</td>
<td>20 days</td>
<td>NA</td>
<td>Dunnett et al., 1981</td>
</tr>
<tr>
<td>Fetal 16-17 days Nigra</td>
<td>Rat</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Augmentation of DA fiber density</td>
<td>10 months</td>
<td>NA</td>
<td>Aguayo et al., 1984</td>
</tr>
<tr>
<td>Adrenal medulla autografts</td>
<td>Rhesus macaque (non-human primate)</td>
<td>6-OHDA unilateral lesion Rhesus macaque</td>
<td>NA</td>
<td>8 months</td>
<td>150 catecholamine neurons</td>
<td>Morihisa et al., 1984</td>
</tr>
<tr>
<td>PC12 cells (rat adrenal medulla pheochromocytoma)</td>
<td>Rat PC12 cell culture</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Decrease in 27% of ipsilateral rotation (amphetamine)</td>
<td>2 weeks</td>
<td>NA</td>
<td>Hefti et al., 1985</td>
</tr>
<tr>
<td>Superior cervical ganglia autografts</td>
<td>Rhesus macaque (non-human primate)</td>
<td>MPTP lesion Rhesus macaque</td>
<td>Increase of 60% of homovanillic acid content in cerebrospinal fluid.</td>
<td>3 months</td>
<td>NA</td>
<td>Itakura et al., 1988</td>
</tr>
<tr>
<td>Fetal 6.5-8 weeks Nigra</td>
<td>Human</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Decrease in 94% of ipsilateral rotation (amphetamine)</td>
<td>19 weeks</td>
<td>1, 600 neurons</td>
<td>Brundin et al., 1988</td>
</tr>
<tr>
<td>Fetal 3 weeks Nigra</td>
<td>Pig</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Abolishment of circling behavior (amphetamine)</td>
<td>17 weeks</td>
<td>Up to 12, 000 neurons</td>
<td>Huffaker et al., 1989</td>
</tr>
<tr>
<td>Fetal 14 days Nigra</td>
<td>Rat</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Decrease in 50% of contralateral rotation (apomorphine)</td>
<td>9 months</td>
<td>3, 400 neurons</td>
<td>Herman et al., 1991</td>
</tr>
<tr>
<td>Fetal 12-13 days Nigra</td>
<td>Mouse</td>
<td>MPTP bilateral lesion Mouse</td>
<td>NA</td>
<td>4 months</td>
<td>2, 600 neurons</td>
<td>Di Porzio &amp; Zuddas, 1992</td>
</tr>
<tr>
<td>Fetal 14 days Nigra expressing PKCβ1 cDNA (second messenger)</td>
<td>Rat</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Decrease in 64% of ipsilateral rotation (amphetamine)</td>
<td>8 weeks</td>
<td>NA</td>
<td>Zhu et al., 1992</td>
</tr>
<tr>
<td>Fetal 13-16 days Nigra</td>
<td>Rat</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Decrease in 70% of contralateral rotation (apomorphine)</td>
<td>3 months</td>
<td>NA</td>
<td>Chung et al., 1993</td>
</tr>
<tr>
<td>Fetal 74 days Nigra</td>
<td>Marmoset (non-human primate)</td>
<td>6-OHDA unilateral lesion Marmoset</td>
<td>Ipsilateral/contralateral rotations per minute ratio of 0.63 vs 5.15 of control lesion</td>
<td>6 months</td>
<td>1, 800 neurons</td>
<td>Amnett et al., 1994</td>
</tr>
<tr>
<td>Superior cervical ganglia (4-week-old culture)</td>
<td>Rat</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Decrease in 40% of contralateral rotation (apomorphine)</td>
<td>4 weeks</td>
<td>NA</td>
<td>Nakao et al., 1995</td>
</tr>
<tr>
<td>Fetal 14 days Nigra mixed with cultured bFGF-producing cells</td>
<td>Rat</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Total reversal of ipsilateral rotation (amphetamine)</td>
<td>8 weeks</td>
<td>2, 800 neurons</td>
<td>Takayama et al., 1995</td>
</tr>
</tbody>
</table>
In spite of the methodological scarcity, these investigations gave the stepping stone towards a clinical approach in PD transplantation and supported the idea that it could be extrapolated to human clinical trials.

3. Transplant therapy in PD patients: A leap forward to a potential successful treatment

Transplantation of dopaminergic cells into the denervated striatum is an experimental approach that was brought to practice to overcome the disadvantages of medications used in the treatment of PD in an attempt to provide the brain with an unlimited source of DA synthesized by the grafted cells. On section 3 we depict the clinical trials that have been taking place until nowadays (Table 2).

The results of the first human trials were published in 1980’s. More than 30 years ago, the major difficulty for performing a transplant was the shortage of dopaminergic cells suitable for transplant and cell-based manipulation techniques were not sophisticated enough at that time. The most logical selection was to employ autologous dopaminergic tissue. Several of the technical and ethical problems, and probably all of the immunologic difficulties, would be solved if catecholamine-producing cells from the patient’s own body were used; this reasoning led two independent groups (Backlund et al., 1985; Drucker-Colin et al., 1988) to investigate if adult adrenal medullary chromaffin cells were able to function as an alternative DA source for transplant. Because the systemic administration of L-DOPA or DA agonist has been successfully applied in the treatment of PD, the logical inference was to expect that adrenal medullary chromaffin cells might act as a chronic pump for delivering DA into the denervated striatum of PD patients.

The first attempt to use autologous medullary chromaffin cell transplant in PD patients was done in Sweden (Backlund et al., 1985), though, the clinical improvement was over a period of one week and one of the patients developed a resistance to L-DOPA treatment.

The first report of significant improvement of symptoms in PD patients after autologous medullary chromaffin cell transplant was done by our (Drucker-Colin) group in México (Drucker-Colín et al., 1988; Madrazo, 1987, 1988). Our team chose a microsurgical transcortical intraventricular approach and implanted autologous adrenal medulla directly into the unilateral caudate nucleus. Although the grafts were unilateral, the improvement was bilateral and symmetric. The direct contact with the caudate nucleus (without the use of a stainless-steel tissue holder that the Swedish group used), exposure to cerebrospinal fluid, and the shorter interval between adrenal dissection and transplantation almost certainly

Table 1. Cell transplant therapy in animal models of PD.

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>Donor</th>
<th>Recipient</th>
<th>Result</th>
<th>Time period</th>
<th>TH neuron count</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engineered grafts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetal 17 weeks retinal pigment epithelial cells attached to microcarriers</td>
<td>Human</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Decrease in 50% of contralateral rotation (apomorphine)</td>
<td>12 weeks</td>
<td>NA</td>
<td>Subramanian et al., 2002</td>
</tr>
<tr>
<td>Fetal retinal pigment epithelial cells attached to microcarriers</td>
<td>Human</td>
<td>MPTP lesion Rhesus macaque</td>
<td>Improvement of 38% in motor tasks (personalized scale)</td>
<td>8 weeks</td>
<td>NA</td>
<td>Doudet et al., 2004</td>
</tr>
</tbody>
</table>
made the difference. However, there were significant risks and disadvantages associated with autologous adrenal transplants; surgical time in a double operation is considered perilous for a PD patient (since most of them are over 60 years) and DA producing cells obtained from adrenal medulla might be not sufficient to compensate the neural DA deficit. These trials were a breakthrough result in the context of transplant therapy in neurodegenerative diseases; the notion that peripheral tissue would be able to survive and integrate within the host’s CNS as well as to induce clinical improvement was not considered to be possible before these studies.

Given the promissory results obtained using adrenal chromaffin cells, which showed variability and technical difficulties; tissue from fetal substantia nigra became to be considered a better option as source of DA cells. Madrazo and Drucker-Colín performed the first transplant of fetal substantia nigra using tissue from cadaveric consent donation (Madrazo et al., 1988). The two transplanted patients showed an improvement of the UPDRS score at 8 weeks post-surgery.

Some years later, in another attempt to improve autolog transplantation of adrenal medullary chromaffin cells, our group took advantage that cultured adrenal chromaffin cells differentiate into neurons in a higher rate when they are stimulated by a low frequency magnetic field (Drucker-Colín et al., 1999). This study proved that adult differentiated adrenal chromaffin cells can be successfully transplanted into the caudate nucleus of a patient with PD and substantially mitigate the clinical symptoms and reduce the intake of L-DOPA medication (see reference video of pre and post-transplanted stages of the surgically treated patient). Postoperative clinical assessments revealed a significant amelioration of visuospatial deficits and a progressive fading of rigidity and akinesia, as well as an improvement in memory tasks; furthermore, a decrease in approximately 70% of L-DOPA intake to ameliorate parkinsonian symptoms was reported.

In spite of the political controversy involving this pioneer studies, the clinical improvement obtained in some patients set off the interest of transplant therapy in PD patients around the world (Deacon et al., 1997; Freed 1990, 2001; Hirsch et al., 1990; Itakura et al., 1997; Kordower 1995, 1996; Lindvall et al., 1990; Peterson et al., 1989; Sawle et al., 1992; Watts et al., 2003) (See table 2).

However, the employment of human fetal dopaminergic neurons as a DA source for transplant is a complicated approach, due to its availability, which is imposed by ethical affairs; for this reason, they have been only employed in some human cases (Freed et al., 1990; Hauser et al., 1999; Kordower et al., 1995; Lindvall, et al., 1990; Madrazo et al., 1988; Mendez et al., 2008; Sawle et al., 1992).

All these initial trials were performed as “open label” (no placebo group as a control), which arose the debate regarding the inclusion of a control placebo group, which is envisaged in general as unethical.

Freed’s group published in 2001 the first double-blind placebo trials using embryonic tissue. Their results were considered disappointing by some researchers and promising by others (Dunnet et al., 2001; Isaason et al., 2001). The controversy is based in the modest recovery observed in the grafted patients compared with the sham group; moreover, the serious dyskinetic side effects observed after several years of transplantation, places this procedure under a doubtful perception of acceptability. However, the fact they found improvement in subjects younger than 60 years, leads to consider better methodologically controlled approaches.

One of the disadvantages in employing this cell source (fetal nigral tissue) is the variability of the results; improvements of parkinsonian symptoms occur in patients who received the
largest amounts of fetal mesencephalic tissue (Hauser et al., 1999; Sawle et al., 1992), whereas in other cases, the transplanted cells did not survive for more than a few months and the patients usually return to preoperative state (Goetz et al., 1991; Hirsch et al., 1990). Additionally, to maintain the fetal tissue viable after transplantation, long-term immunosuppressive treatment is needed. Nonetheless, without a doubt the major limiting factor of this approach is the difficulty in obtaining a sufficient amount of human viable fetal tissue.

Dejectedly, as has been observed in those studies, there are some patients that clearly demonstrate improvement and others that do not show any recovery; however, it is still not possible to unravel this mystery of selectivity. Within the possible mechanisms accounting for these changes, there is patient age, transplant technique, cell graft source and as the most probable candidate the immune system; which remains the most formidable barrier to transplantation as a routine medical treatment (Freed et al., 2004). The immune system has developed elaborate and effective mechanisms to combat foreign agents and we are now in pursuit of elucidating them.

The successes of human to human organ transplantation, coupled with severe limitations in the availability of human organs, propelled research into the use of non-human organs in the 1990’s. The domesticated pig was the preferred donor at the time. In an audacious attempt to replace fetal tissue, surgery to transplant brain tissue from pig fetuses into the caudate-putamen of individuals diagnosed with PD was performed. They reported a clinical improvement of rigidity in a period of 7 months and that autoimmune processes did not become established in the patients (Deacon et al., 1997). Unfortunately, safety and efficacy have not yet been clearly demonstrated, and information is still preliminary to warrant this therapy. Current opinion is that the risk of transfer of a significant infectious microorganism from the pig to the human recipient of a xenograft is small and ethically acceptable if the transplant will be highly beneficial to the host. However, pig specific pathogens are considered to be of potential risk, thus, limiting the use of pig xenografts. Clinical xenotransplantation is becoming feasible and attractive as a routine therapy, nonetheless, some attention should be set on this matter.

In another attempt to evade the host immune response after transplantation, human retinal pigment epithelial (hRPE) cells were taken into consideration. As mentioned previously, experimental studies suggested that RPE cells were a suitable cell type to serve as a potential enduring source of L-DOPA for implantation into the striatum of animal models of PD (Subramanian et al., 2002). These implants ameliorated the motor deficits in rodent and non-human primate models of PD and immune suppression was not required even when transplants were made from one species to another (Doudet et al., 2004; Subramanian et al., 2002).

Those results were taken into account in a novel tissue engineering strategy; hRPE cells were attached to gelatin microcarriers (spheramine™) and transplanted into the putamen contralateral to the more symptomatic side of patients with Parkinson disease (Watts et al., 2003). They reported an average improvement of 48% in motor score 12 months after implantation evaluated through the Unified Parkinson’s Disease Rating Scale (UPDRS) with the patient in the “off” state, which was sustained for 24 months. Improvement was also observed in activities of daily living, quality of life, and motor fluctuations.

However, in a more recent work, a post-mortem study of a patient enrolled in a similar clinical trial reported that only 118 cells from the transplant endured (estimated 0.036% survival) and that clinical improvement was not observed (Farag et al., 2009).
engineered tissue approach is still an emerging therapy alternative for PD and the existing body of literature is still inadequate to allow conclusions regarding this procedure. Concerns related to small sample sizes and a limited number of controlled trials are joined with a wide array of methodological issues with the procedure itself. We have no doubt that better procedures will arrive alongside the advances in tissue development.

The leading inquiry raised by transplant therapy hitherto is the variability in functional recovery either in animal models and human patients. In human studies, this unevenness is not only seen between different trials, but also within groups of PD patients transplanted in the same assay. In order to attend such matters, a comprehensive clinical trial must be done, one that includes a meticulous analysis of patient selection (e.g., age, level of PD), knowledge of the number of DA neurons transplanted, type of surgery, follow up of the patient before surgery, monitoring of the clinical improvement after grafting, analysis of cell survival and neuron functionality. A successful cell therapy must settle advantages over current treatments for lessening motor symptoms in PD patients. Cell replacement should provide long-lasting, major improvements of mobility and no manifestation of dyskinesias without the need of further therapeutic interventions, an uneasy task that most likely will not be achieved by using solid fetal ventral midbrain grafts. This is suggested in the recent report from Mendez (Mendez et al., 2008), who transplanted fetal ventral midbrain as cellular suspension. The analysis did not reveal development of diskynesias in the transplanted PD subjects in a period spanning from 9 to 14 years; moreover, the postmortem analysis of the transplanted patients’ brains did not show any sign of degeneration (alpha synuclein, ubiquitin), and only a minimum of microglial reaction from the host was found. In contrast, in the two double blind placebo trials, where solid pieces of fetal midbrain tissue were used, severe microglial reaction was found and diskynesias were also observed (Freed et al., 2001; Olanow et al., 2003). Mendez’s results supports a new “hope” in medical treatment for PD patients based in stem cell tissue procedures, after the setback given by the report from Kordower of transplant analysis following 14 years after the procedure (Kordower et al., 2008), in which the postmortem tissue revealed that the grafted cells in the striatum were affected by a neurodegenerative mechanism, suggesting that the neurodegenerative process was not exclusive of the SNPc. Taken together, the present observations suggest that the methodological procedures and not the cell source, are directly involved in the limited success of transplant therapy that employs solid portion of fetal ventral tissue.

Currently, there are various ongoing stem cell based clinical trials registered by the National Institutes of Health (NIH): (http://www.clinicaltrials.gov/ct2/results?term=transplant +parkinson), and new assessments are being developed by the Transeuro consortium (Allan et al., 2010) (http://www.transeuro.org.uk/index.html). The European group coordinated by Dr. Roger Barker is planning highly controlled studies in the near future (2012). Interestingly, the cells to be employed in these trials will be obtained from the midbrain of human fetuses, the same tissue we used the first time more than 3 decades ago. Again, as in past, the idea still creates controversy between those who concur with such methods and those who are unfavorable to it due to the limited success percentage (Holden, 2009).

We expect that the new trails should provide better motor recovery to the ones observed in previous assays. Even though behavioral improvement is seen in experimental transplant trials, going from 30% to total reversal of circling behavior (Tables 1 and 3), the motor symptoms observed in grafted human subjects when analyzed by UPDRS do not surpass 60% of clinical improvement (Table 2 and Dunnet et al., 2001).
<table>
<thead>
<tr>
<th>Cell Source</th>
<th>Donor</th>
<th>Implant Zone</th>
<th>Result</th>
<th>Time period</th>
<th>TH neuron count</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous adrenal medullary tissue</td>
<td>Human</td>
<td>Striatum (Putamen)</td>
<td>Improvement of rigidity</td>
<td>1 week</td>
<td>NA</td>
<td>Backlund et al., 1985</td>
</tr>
<tr>
<td>Autologous adrenal medullary tissue</td>
<td>Human</td>
<td>Lateral Ventricle</td>
<td>Disappearance of rigidity and akinesia</td>
<td>5 months</td>
<td>NA</td>
<td>Madrazo et al., 1987</td>
</tr>
<tr>
<td>Autologous adrenal medullary tissue</td>
<td>Human</td>
<td>Striatum (Caudate)</td>
<td>Decrease in 50% of L-DOPA dose</td>
<td>1 year</td>
<td>NA</td>
<td>Drucker-Colin et al., 1988</td>
</tr>
<tr>
<td>Autologous adrenal medullary tissue/fetal 13 weeks substantia nigra tissue</td>
<td>Human</td>
<td>Lateral Ventricle</td>
<td>Disappearance of rigidity and akinesia</td>
<td>2 years</td>
<td>NA</td>
<td>Madrazo et al., 1988</td>
</tr>
<tr>
<td>Autologous adrenal medullary tissue</td>
<td>Human</td>
<td>Striatum (Putamen)</td>
<td>Improvement of rigidity</td>
<td>2 weeks</td>
<td>Necrotic tissue</td>
<td>Peterson et al., 1989</td>
</tr>
<tr>
<td>Autologous adrenal medullary tissue</td>
<td>Human</td>
<td>Striatum (Caudate)</td>
<td>None</td>
<td>4 months</td>
<td>Necrotic tissue</td>
<td>Hirsch et al., 1990</td>
</tr>
<tr>
<td>Fetal 45-55 days Nigra tissue</td>
<td>Human</td>
<td>Striatum (Caudate and Putamen)</td>
<td>Improvement of Hoehn–Yahr scale from 3.71 to 2.5</td>
<td>4 years</td>
<td>N.A.</td>
<td>Freed et al., 1990</td>
</tr>
<tr>
<td>Fetal 8-9 weeks Nigra tissue</td>
<td>Human</td>
<td>Striatum (Caudate)</td>
<td>Increase in 130% of [18F]-DOPA intake in PET scan. Increase of L-DOPA effectiveness from 2 to 14 hours</td>
<td>5 months</td>
<td>NA</td>
<td>Lindvall et al., 1990</td>
</tr>
<tr>
<td>Autologous adrenal medullary tissue</td>
<td>Human</td>
<td>Striatum (Caudate)</td>
<td>Clinical improvements of 19% in UPDRS score</td>
<td>2 years</td>
<td>NA</td>
<td>Goetz et al., 1991</td>
</tr>
<tr>
<td>Fetal 8-9 months Nigra tissue</td>
<td>Human</td>
<td>Striatum (Putamen)</td>
<td>Decrease in 60% of L-DOPA dose</td>
<td>18 months</td>
<td>NA</td>
<td>López-Lozano et al., 1991</td>
</tr>
<tr>
<td>Fetal 6.5-9 weeks Nigra tissue</td>
<td>Human</td>
<td>Striatum (Putamen)</td>
<td>Improvement of UPDRS from 78 to 49.5</td>
<td>18 months</td>
<td>210,000 neurons</td>
<td>Kordower et al., 1995/1997</td>
</tr>
<tr>
<td>Superior cervical ganglia autografts (stellate ganglion)</td>
<td>Human</td>
<td>Striatum (Putamen)</td>
<td>Reduction of 33% in the time taken to perform motor task. No changes in UPDRS score</td>
<td>3 years</td>
<td>NA</td>
<td>Itakura et al., 1997</td>
</tr>
<tr>
<td>Fetal 9 weeks Nigra tissue</td>
<td>Pig</td>
<td>Striatum (Caudate and Putamen)</td>
<td>Improvement of rigidity</td>
<td>7 months</td>
<td>NA</td>
<td>Deacon et al., 1997</td>
</tr>
<tr>
<td>Adrenal medullary tissue differentiated to DA cells in 60Hz magnetic field</td>
<td>Human</td>
<td>Striatum (Caudate)</td>
<td>Decrease in 70% of L-DOPA dose</td>
<td>1 month</td>
<td>NA</td>
<td>Drucker-Colin et al., 1999</td>
</tr>
<tr>
<td>Cell Source</td>
<td>Donor</td>
<td>Implant Zone</td>
<td>Result</td>
<td>Time period</td>
<td>TH neuron count</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>------------------------</td>
<td>-------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>-------------</td>
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<td>-------------------------------</td>
</tr>
<tr>
<td>Fetal 6-9 weeks Nigra tissue</td>
<td>Human</td>
<td>Substantia Nigra Pairs compacta</td>
<td>Clinical improvements of 32% in UPDRS score</td>
<td>20 months</td>
<td>80,000 neurons</td>
<td>Hauser et al., 1999</td>
</tr>
<tr>
<td>Fetal Nigra tissue</td>
<td>Human</td>
<td>Striatum (Putamen)</td>
<td>Increase in 40% of [18F]-DOPA intake in PET scan. Clinical improvements of 30% in UPDRS score</td>
<td>1 year</td>
<td>30,000 neurons</td>
<td>Freed et al., 2001</td>
</tr>
<tr>
<td>Fetal Nigra tissue</td>
<td>Human</td>
<td>Striatum (Putamen)</td>
<td>Clinical improvements of 15% in UPDRS score</td>
<td>2 years</td>
<td>NA</td>
<td>Olanow et al., 2003</td>
</tr>
<tr>
<td>Suspended fetal ventral midbrain cells from 6-10 weeks</td>
<td>Human</td>
<td>Striatum (Putamen)</td>
<td>Increase in 185% of [18F]-DOPA intake in PET scan. Clinical improvements of 60% in UPDRS score</td>
<td>1 year</td>
<td>NA</td>
<td>Mendez et al., 2008</td>
</tr>
<tr>
<td>Adult neural stem cells grown as neurospheres</td>
<td>Human</td>
<td>Striatum (Putamen)</td>
<td>Increase in 30% of [18F]-dopa intake in PET scan. Clinical improvements of 80% in UPDRS score</td>
<td>1 year</td>
<td>NA</td>
<td>Lévesque et al., 2009</td>
</tr>
<tr>
<td>Autologous bone marrow-derived mesenchymal stem cells</td>
<td>Human</td>
<td>Sublateral ventricular zone</td>
<td>Clinical improvements of 22% in UPDRS score</td>
<td>3 years</td>
<td>NA</td>
<td>Venkataramana et al., 2010</td>
</tr>
<tr>
<td>Engineered grafts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinal Pigment Epithelial cells attached to microcarriers</td>
<td>Human</td>
<td>Striatum (Putamen)</td>
<td>Clinical improvements of 48% in UPDRS score</td>
<td>1 year</td>
<td>NA</td>
<td>Watts et al., 2003</td>
</tr>
<tr>
<td>Retinal Pigment Epithelial cells attached to microcarriers</td>
<td>Human</td>
<td>Striatum (Putamen)</td>
<td>None</td>
<td>6 months</td>
<td>118 cells</td>
<td>Farag et al., 2009</td>
</tr>
</tbody>
</table>

Table 2. Cell and stem cell transplant therapy in patients with PD.

4. Stem cell transplantation: Is it the idyllic approach?

Early research gazed at ventral mesencephalic fetal dopaminergic tissue for transplantation in animal models of PD and PD patients with partial success. Nonetheless, while fetal primary tissue showed promise, the widespread clinical application of this approach is considered limited due to the matter of rejection and low cell survival. The search for
alternative sources able to circumvent at least some of the problems inherent to fetal tissue is actually undergoing. Since the advent of stem cells, they have been proposed as potential candidates to generate dopaminergic mesencephalic neurons and replace the cell loss that takes place in PD. Stem cells are of an undifferentiated nature and possess an extend capacity to proliferate, as well they are capable to endow cells with the same sort of undifferentiated state. These cells are sorted in accordance to their intrinsic capacity to generate different cellular types, ability that diminishes throughout embryonic development. They are considered totipotent when they are able to originate any kind of embryonic or extra-embryonic cell (few days embryo) (Thomson & Odorico, 2000). Further in embryonic development, in the blastocyst stage, cells of the inner cell mass (ICM) have the potential to contribute to the three embryonic germinal layers, ectoderm, mesoderm and endoderm, and they are classified as pluripotent. Finally, when the embryo evolves to the gastrula stage, the ICM cells have already differentiated and compromised to a specific lineage, based on the elapsed time and location within the embryo. These cells are multipotent and they are present in the embryo as well as in the adult organism; an example is the neural stem cells (NSC) discussed below. We should classify stem cells in three broad groups: Adult neural stem cells (neural stem cells and mesenchymal), embryonic stem (ES) cells and the recently obtained induced pluripotent stem (iPS) cells. All of them have been considered in greater or lesser degree accordingly to their specific characteristics as a potential source of dopaminergic cells. With this wide diversity of cells available for transplant, the question directly arising is, “is there an idyllic stem cell to be employed as source of dopaminergic neurons?” said it in other way, “is there a cell capable of long-term survival, steady release of DA, integration into the host brain and therefore induce functional benefits without side effects?” In this section we will describe the properties and capacity of stem cells from different origin to become dopaminergic neurons, with the purpose of elucidating that question. Additionally, we will describe the results of the first stem cell transplant experiments performed in animal models of PD and the few trials implemented in humans.

4.1 Adult neural stem cells
Several studies indicated the existence of dividing cells in the CNS (Altman, 1969) but the discovery and isolation of a subtype of multipotent cells, the NSC, in specific regions of the mice adult brain (Reynolds & Weiss, 1992) and later in the human brain (Eriksson et al., 1998) were the events that clearly revealed that the adult CNS posses an inherent plastic capability. This instigated the relentless pursue to replace the cellular loss that takes place in the CNS after injury or neurodegenerative diseases. NSC are self-renewing progenitors specified to give rise only nervous tissue-specific cell types, neurons, glia and oligodendroglia (Reynolds and Weiss, 1996). In adult rodent brain, the SVZ and the dentate gyrus of the hippocampus contain NSC population with permanent capacity of proliferation (Doetsch et al., 1999; Gage, 2000). These brain areas with potential to generate new neurons are defined as neurogenic niches. The advantage of containing an adult source of stem cells is the possibility of avoiding the use of human fetal tissue and embryonic derived stem cells for replacement therapy in neurodegenerative diseases.

4.1.1 NSC from adult subventricular zone (SVZ)
Neural stem cells from the SVZ move in chain migration along the rostral migratory stream (RMS) and differentiate into periglomerular interneurons at the olfactory bulb throughout
the rat lifespan (Doestch et al., 1999). The functional relevance of permanent replacement of periglomerular neurons in the rodent brain is attributed to the olfactory adjustment to odor changes in the environment.

Noteworthy, stem cells in the SVZ are able to respond to adverse damage in the brain. An increase in proliferation is observed under traumatic conditions such as acute stroke (Arvidsson et al., 2002; Parent et al., 2002) or in chronic stroke after being stimulated by transforming growth factor alpha (TGFα) (Guerra et al., 2009). Conversely, in animal models of PD, decreased proliferation of the progenitors in the SVZ takes place (Holginger et al., 2004). Interestingly, a simultaneous increase of Paired box gene 6 (Pax6) dopaminergic interneurons in the periglomerular layer is also occurring (Winner et al., 2006). The same increase of TH+ cells is observed in the olfactory bulb of postmortem tissue in PD patients (Huisman et al., 2004).

The reasons to consider the adult multipotent progenitors of the SVZ as an option to regenerate the dopaminergic population in the affected striatum of PD patients are their commitment to differentiate into dopaminergic neurons in the olfactory bulb and that they remain responsive to different signals.

However, it is well known that different set of transcription factors determine the correct dopaminergic fate in the olfactory bulb and SNPC. In the olfactory bulb, transcription factors such as the ETS transcription factor Er81 (Er81), Pax6 and Distal-less homeobox 2 (Dlx2), regulate the terminal differentiation of periglomerular dopaminergic neurons (Brill et al., 2008; Cave and Baker 2009; Hack et al., 2005; Kohwi et al., 2005). Instead, the initial specification of dopaminergic mesencephalic neurons from the SNPC is regulated by the LIM homeobox transcription factor 1 (Lmx1a) and the homeobox transcription factor 1 (Msx1) (Andersson et al., 2006). This evidence suggests that the neuroblasts arising from the SVZ do not develop into A9 dopaminergic neurons, the cells mainly affected in PD (German et al., 1992). Additionally, when NSC are expanded in culture as floating cellular aggregates in the neurospheres assay they mainly generate glial cells (Storch et al., 2004). Together, these results on animal models suggest that adult NSC arising from the SVZ should not be an optimal selection for cell therapy in PD.

4.1.2 Neural stem cells-fetal derived transplants.
Different protocols in vitro have been developed to generate dopaminergic neurons from NSC (for review see Deirborg et al., 2008). In spite the expansion process of NSC, it decreases their potential to differentiate into dopaminergic neurons (Ptak et al., 1995); in rats, it was demonstrated that fetal ventral mesencephalic (VM) precursor cells have the potential to proliferate and differentiate into dopaminergic neurons in culture when stimulated by the growth factor FGF-2 (Studer et al., 1998). An important fact involving the use of the expanded fetal neural stem cells is the low survival observed after grafting the lesioned striatum (around 3-5%), therefore, the improvement of behavioral deficits is not great compared to the one obtained with primary fetal cells (not amplified) (Brundin et al., 1988). Even thought fetal neural stem cells are not the most adequate source for transplant, they could be considered a better option than NSC from the SVZ.

4.1.3 Neural stem cells-adult derived transplant
NSC from adult tissue have been employed in a relevant clinical trial, neural stem cell-derived neurons were isolated from cortical and subcortical tissue and expanded in vitro for
several months. Nine months after harvesting, autologous cell suspensions containing differentiated dopaminergic and GABAergic neurons were microinjected unilaterally in a patient with advanced Parkinson’s disease. Over the next 3 years, the overall UPDRS improved by 80%. However, at five years post-operatively, clinical motor scores returned to baseline (Table 2) (Lévesque et al., 2009).

Even though this result is not the most promising one, it laid the foundation for the development of autologous neural stem cell based therapy in PD patients and pointed out that this source represents another option for cell replacement therapy, avoiding the employment of embryonic tissue.

4.1.4 Mesenchymal stem cells (MSCs)

Efforts in employing non-fetal stem cells for transplant therapy in PD are also currently undergoing. Venkataramana et al. performed a unilateral transplantation of autologous bone marrow-derived mesenchymal stem cells (BM-MSCs). The BM-MSCs were transplanted into the sublateral ventricular zone by stereotaxic surgery. The transplanted patients showed a clinical improvement of 23% in their UPDRS score. Moreover, a subjective improvement was found in symptoms like facial expression, gait, and freezing episodes (Venkataramana et al., 2010). These results indicate that this new protocol seems to be safe, and no serious adverse events occurred after stem-cell transplantation in PD patients, additionally, no fetal tissue was needed to obtain human stem cells. However, more efforts should be done to improve these stem cell-based techniques in experimental models of PD before using them in PD patients.

As far as we know, this is the last report of a clinical trial in PD patients. Taking this and all of the previous works done for transplant therapy in PD patients, we can state that the international quest for the best transplant therapy should not have been taken with such haste; a more detailed analysis of graft survival and long term clinical improvement are considered necessary. Relevant concerns prevail about methodology aspects, and also about the most debated complication of cell therapy in PD, the occurrence of post-operative graft-induced dyskinesias in the majority of the clinical protocols.

4.2 Embryonic stem cells: The most promising source of dopaminergic neurons

The information described above shows that even though fetal midbrain precursors have capacity to proliferate and differentiate into dopaminergic neurons, the efficiency of this process in culture is low, therefore, restricting their potential as donor tissue. ES cells instead have the unique ability to self-renew indefinitely while maintaining the potential to give rise to all cell types in the human body. These two properties of ES cells make them gain a remarkable interest as promising tools for regenerative medicine, specially in PD transplant therapy. Another advantage that propelled the use of stem cells is that genetic manipulations has became easily practicable in the last few years.

Nevertheless, currently there are three major aspects limiting the success of stem cell therapy; one being the low number of stem cells sources with the potential to differentiate into the mesencephalic dopaminergic phenotype; the limited survival of grafted cells transplanted in both animal models and humans; and the most important, the danger of teratomas.

In this section, we summarize the different attempts to increase the number of stem cells using basically 3 different culture systems (feeder stromal cells, embryoid bodies and neural
rossetes) to induce differentiation towards a dopaminergic phenotype. Evaluation of survival, integration and function of ES-dopaminergic cells after transplantation in animal models of PD is also described.

4.2.1 Mouse Embryonic stem (mES) cells
In 2000, Kawasaki et al. performed the first grafted study of mice ES-dopaminergic derived cells into the mouse striatum that had been previously treated with 6-OHDA. The system used to derive TH mesencephalic-type neurons consisted in culturing ES cells onto the bone marrow stromal cell line PA6, which they identified to have the feature (by unknown mechanism) of promoting specific dopaminergic differentiation. The number of TH neurons obtained by this co-culture method is significantly higher (~16% of the total cells) to the five stages protocol reported in the same year by Lee et al. and collaborators (Lee et al., 2000). Two weeks after transplantation, 22% of the grafted TH-positive neurons survived and even though the improvement of rotational behavior after transplant was not analyzed, they confirmed that ES-dopaminergic cells are capable of being transplanted into a parkinsonian model and survive, which laid the interest to explore further possibilities for therapeutic application (Kawasaki et al., 2000).

In continuance work, other research groups have been improving culture protocols in order to increase the number of dopaminergic neurons to be grafted. In 2002, the first transplant of mES cells took place, Kim et al. reported that ~78% of dopaminergic neurons could be derived from mES cells in vitro. The highest number of TH cells reported until now was promoted by the overexpression of the nuclear receptor related-1 (Nurr1), a transcription factor relevant in the induction of mesencephalic precursors into dopamine neurons (Wallen et al., 1999). Interestingly, a remarkable behavioral improvement after grafting was observed; the transplanted parkinsonian rats presented a total reversal of amphetamine-induced rotational behavior 8 weeks after transplant (Kim et al., 2002). The behavioral enhancement correlates with the evaluated release of dopamine and functional synapses of the transplanted cells. Additionally, no tumor formation was found.

That same year, Bjorklund et al. followed an opposite transplant approach. The group grafted ES cells on embryoid body (EB) stage. It is important to mention that EBs are aggregated of cells with spherical shape that differentiate stochastically from ES cells under specific in vitro conditions, they have the inherent property of recapitulate embryonic development and were developed the first time by Lee in a 5 stage protocol (Lee et al., 2000). Rats transplanted with EB presented a decrease in 46% of amphetamine-induced rotation 9 weeks after transplantation (Bjorklund et al., 2002). The reasoning of the improvement in the motor asymmetry is that the ES cell-derived neurons released dopamine in sufficient amount when they were stimulated by amphetamine. However, the number of TH+ neurons produced after grafting was not studied. They instead analyzed the functional activity of ES cells by PET imaging, finding high similarity to that observed in transplanted dopaminergic midbrain fetal neurons. Unfortunately, in contrast to the study realized by Kim et al., 25% of the animals developed tumors, a result expected given the fact that not all of the grafted cells were in a differentiated state.

To improve the therapeutic potential of ES cells Barberi et al. implemented a faster and simpler culture method. Their protocol consisted in the induction of the differentiation of mice ES cells into dopaminergic neurons using the feeder stromal cell line (MS5). They found that nuclear transfer-derived ES dopaminergic neurons following this protocol and
transplantation into the striatum of parkinsonian mice provoked a decrease in 80% of circling behavior; additionally, some animals contained up to 40,000 transplanted TH+ neurons 8 weeks after transplant (Barberi et al., 2003).

In another effort to obtain functional DA neurons derived from ES cells but without any genetic modification, Rodríguez-Gómez et al. generated CNS progenitor populations from mES cells that were later expanded and promoted to differentiate into dopaminergic neurons in the presence of mitogen and specific signaling molecules. Mitogen withdrawal from the growth medium was done after expressing Engrailed 1 (En1), Paired box gene 2 (Pax2), and Orthodenticle homeobox 2 (Otx2) specific neuronal transcription factors, hence, achieving differentiation (Rodríguez-Gómez et al., 2007). They also described that in the grafted animals, PET imaging showed that the number of postsynaptic DA D2 receptors was normalized in the host striatum. Additionally, microdialysis in grafted animals displayed that dopaminergic transplanted neurons release was induced by depolarization and pharmacological stimulants. Their data suggest that ES cell-derived neurons release DA in a physiological manner and that reuptake postsynaptic responses remains after implantation.

On the other hand, to optimize the procedure for generation of mesencephalic dopaminergic neurons from ES cells, Jönsson et al. elaborated a protocol to determine the optimal stage of development of cells used for grafting. By means of fluorescence-activated cell sorting procedures, they isolated DA precursors from mouse ventral mesencephalon in two defined stages of differentiation, at 10.5 and 12.5 embryonic days, when the dopaminergic mesencephalic neurons are arising. After transplantation into the striatum of 6-OHDA-lesioned rats, the histological analysis showed that TH-expressing cells poorly survived sorting and transplantation; however, this study demonstrated that the differentiation state of the progenitors is important. The transplanted neuroblasts originate more TH+ cells when obtained from an early state of differentiation (Jönsson et al., 2009). These outcomes have inferences for recent efforts to develop well-characterized stem cell-derived mesencephalic DA progenitor cell preparations for future cell therapy.

Even though the 6-OHDA lesioned rat is a great model of PD for analyzing ES cell transplant therapy, before considering preliminary transplantation trials in PD patients, experiments on non-human primates are quintessential. With this in mind, Takagi et al. generated neurospheres composed of neural progenitors from monkey ES cells cultured on PA6 stromal feeder cells and transplanted them into the putamen of MPTP lesioned monkeys. They reported a significant behavioral recovery and an average survival of 2,100 TH+ transplanted neurons 14 weeks after transplantation; additionally, PET imaging revealed a 50% increase in 18F-fluorodopa uptake. This study demonstrated that the transplanted ES cells functioned as DA neurons and alleviated the motor symptoms in a parkinsonian non-human primate (Takagi et al., 2005); furthermore, they demonstrated that ES cell transplant therapy might be an appropriate treatment alternative to human PD patients.

4.2.2 Human ES cells

After Thomson isolated stem cells from human embryo (Thomson et al., 1998), the next boundary to overcome was to generate neural progenitors derived from human ES (hES) cells. In an innovating experiment, undifferentiated hES cells were plated on fresh mitotically inactivated feeders and cultured for 8 days to induce neural differentiation, these progenitors were transplanted into the striatum of parkinsonian rats. The grafts survived for at least 12 weeks, managed to differentiate in vivo to DA neurons and improved circling
behavior with a 45% reduction of amphetamine-induced ipsilateral rotation (Ben-Hur et al., 2004). Induced dopaminergic differentiation from hES was shortly addressed by other groups with different culture approaches but with similar relatively successful outcomes (Brederlau et al., 2006; Chiba et al., 2008; Cho et al. 2008; Roy et al., 2006).

Roy used a combined strategy to improve dopaminergic neuron amounts. Sonic hedgehog (Shh) and fibroblast growth factor 8 (FGF-8) were co-cultured with telomerase-immortalized hES cells derived from human fetal midbrain astrocytes in order induce a dopaminergic neuronal fate. After achieving a high number of TH+ cells specific for the A9 dopaminergic lineage (colocalizing with the G protein–gated inwardly rectifying K+ channel, Girk2), they were transplanted into the striatum of 6-OHDA lesioned parkinsonian rats. The authors reported that the dopaminergic implants generated a significant, substantial and long-lasting restitution of motor function. Nonetheless, the grafts displayed increasing cores of undifferentiated mitotic neuroepithelial cells, which can turn tumorigenic (Roy et al., 2006). These data dictate the need for extreme caution in developing a clinical application of hES cell-derived grafts, given their potential for undifferentiated expansion, yet they also proved that TH+ neurons can be obtained and/or induced from a diverse source of cells.

Chiba et al. followed a different method to improve hES cell transformation to phenotypically stable DA neurons. They blocked the effect of the neuronal differentiation promoter Noggin by means of the bone morphogenic protein (BMP) antagonist. With this strategy they found that BMP inhibitor Noggin increased production of DA neurons from hES cells differentiated on PA6 stromal cells. In addition, these DA neurons survived transplantation and led to behavioral improvement in parkinsonian rats 4 weeks after grafting (Chiba et al., 2008). These neurons derived from hES cells may not be suitable for eventual transplantation into PD patients (due to a triple trisomy created by continuous passaging), but with this work, they demonstrated that Noggin has a critical role for determining midbrain dopaminergic phenotype at an early stage in ES cell differentiation, which will be of great value for future research in dopaminergic cell engineering.

The recent generation of dopaminergic mesencephalic neurons obtained from human ES cells by employing spherical neural masses (SNMs), also called neural rosette cells, is considered the protocol that allows to yield the higher number of dopaminergic neurons in cell culture (roughly 66%) (Cho et al., 2008). SNMs are columnar epithelial structures that represent a novel NSC type, distinct and more primitive than those previously characterized from other NSC stages (Elkabetz et al., 2008; Pankratz et al., 2007). NSC in neural rosettes mimic the neural plate stage and can be directed towards a dopaminergic mesencephalic lineage by employing the signaling molecules Shh and FGF-8 (Elkabetz et al., 2008; Cho et al., 2008). However, whether the fully differentiated DA cells obtained from this novel protocol have an ameliorative effect on parkinsonian rodent models or PD patients still remains to be determined. Cho and collaborators decided to transplant cells originated from neural rosetes in the initial stage of dopaminergic neuronal specification into the lesioned striatum of parkinsonian rats in order to avoid a low survival rate. In this specific time point, approximately 50% of the cells were TH+ and immunohistochemical analysis demonstrated that only 2.7% of the grafted neurons were TH+ 12 weeks after transplantation. Despite this low cell number, a significant behavioral recovery was observed in 3 different motor behavioral tests. Although this stem cell source is promissory due to the high number of yield DA cells without the need of genetic modifications or employment of feeder cells; this technique stills needs to circumvent the problem of low survival ratio, which is generally
observed when terminal differentiated cells are grafted. Additionally, in order to generate an efficient long-term culture (30 to 40 days), experimented handling and dedicated efforts are required. These results also point out the necessity of better and rational procedures to direct cell specification to a desired phenotype \textit{in vitro}.

The bulk of results suggest ES cell transplantation is approaching the point of technical practicability toward clinical therapy for PD. hES cell transplantation has the potential to be considered one of the most promising therapies for PD treatment; however, this excitement is gradually fading as a result of political and ethical concerns. Amongst the limiting methodological factors, is the difficulty to maintain normal karyotype and to avoid tumor formation. Furthermore, the differentiation rate of ES cells towards a dopaminergic lineage is not high enough to obtain sufficient dopaminergic cells for transplantation.

4.3 Induced pluripotent cells: The most recent breakthrough in stem cells field

Induced pluripotent (iPS) cells are derived from somatic differentiated cells by overexpression of specific transcription factors, which induces cell reprogramming. They are fairly similar to ES cells in terms of self-renewal and pluripotency, which provides them with the potential to differentiate into any cell type in the organism.

iPS cells were obtained for the first time in 2006 by Yamanaka’s group; they were acquired from embryonic and adult mouse fibroblasts reprogrammed to a pluripotent-like state by viral transduction of four transcription factors: Klf4, cMyc, Oct3/4 and Sox2. The obtained cells demonstrated to possess typical ES cells properties (Takahashi et al., 2006); however, they did not form effective fertile chimeras, meaning that the ability to transmit genotype through the germline was not achieved, this being one of the most important properties that defines a pluripotent cell. This was later rein-vindicated in 2007 in a parallel work by Jaenisch’s and Yamanaka’s groups, they successfully reprogrammed fibroblasts and obtained viable chimeras (Okita et al., 2007; Wernig et al., 2007); nonetheless, they emphasized that the transduction of c-Myc should be avoided in iPSC reprogramming, due to its tumorigenic capacity.

At the end of 2007, and almost simultaneously, Takahashi and Yu were able to manufacture iPS cells from human dermal fibroblast. Takahashi enforced the expression of the same transcription factors employed in the previous study (Klf4, cMyc, Oct3/4, Sox2); while Yu et al., demonstrated that other two transcription factors, Nanog and LIN28 (besides Oct3/4, Sox2) were also relevant in cell reprogramming (Takahashi et al., 2007; Yu et al., 2007). One year later, iPS cells were derived from human fetal tissue, neonatal and adult somatic cells by employing the same combination of genes used by the pioneer team, they also reported that c-Myc only increases the efficiency of reprogrammation, but it is not essential for the establishment of a pluripotency state, suggesting that c-Myc could be nonessential for reprogrammation (Park et al., 2008). Taken together, these results reveal that iPS cells are an attractive resource for replacement therapy, given the fact that its use would overcome the technical and ethical difficulties of ES cells by avoiding the use of human embryos, circumventing transplant rejection and therefore avoiding the use of immune suppressants. However, concerns for the transduction of the oncogen c-Myc were still latent and attempts to remove it from the transcription factor cocktail were in progress.

Later in the same year, it was demonstrated that c-Myc is absolutely expendable to obtain viable iPS cells, either from mice and human samples (Nakagawa et al., 2008). With the removal of this specific proto-oncogen, the possibility of tumor formation was reduced, and therefore, research on possible clinical applications achieved important notoriety.
iPS cell lines obtained from patients afflicted with different diseases have been developed with the purpose of investigating the therapeutic potential of autologous transplants, but also to offer an unprecedented opportunity to recapitulate pathologic conditions in vitro (Gunaseeli et al., 2010; Park et al., 2008). Neurodegenerative diseases are particularly taken into consideration, since they are secondary to a relatively selective loss of neurons, for that reason, the quest for replacing the neuronal loss of a specific population employing iPS cells is currently undergoing in a similar way that occur for ES cells.

The iPS cells neuronal differentiation in vitro assays have been successful and were achieved by employing established ES cell differentiation protocols. The first dopaminergic differentiation was obtained from neural precursors derived from mice iPS cells. These cells were grafted into the mice fetal encephalon (chimerical brain) and revealed a high capacity for migration, differentiation and integration within different brain areas. The iPS-derived dopaminergic neurons generated in this study were grafted in the 6-OHDA rat model of PD, such transplants improved significantly circling behavior stimulated by amphetamine (Wernig et al., 2008). However, it was reported that the presence of undifferentiated cells in the graft promoted tumor formation; this fact contributed to the idea that obtaining a purified population is essential for a satisfactory transplant outcome.

It has been proved that pluripotent cell production can be obtained from patients suffering from other neurodegenerative disease. Dimos et al. developed iPS cells from patients with amyotrophic lateral sclerosis (ALS), which was effectively differentiated in vitro to motoneurons, the degenerated cells that are accountable for the development of ALS (Dimos et al., 2008). This corroborated that iPS cells patient-specified manufacture is a viable option and possess certain advantages over ES cells since iPS cells evade the ethical and methodological difficulties presented in ES cells production.

A new study performed in 2009 widened the possibility of inducing patient-specified neural differentiation in vitro into iPS cells engineering. Jaenisch’s group managed to generate fibroblast-derived iPS cells from 5 idiopathic PD patients, with the advantage that once the cellular reprogramming employing three transcription factors (Oct4, Klf4 y Sox2) was achieved, they were able to remove all viral vectors using Cre-recombinase (iPS cells factor-free). These cells maintained all the properties of a pluripotent cell and were capable to induce neuronal differentiation towards a dopaminergic phenotype in vitro; additionally, they have the advantages of being a free viral transcription factor cell line and that overall gene expression patterns were more similar to ES cells than carrying-factor iPS cells (Soldner et al. 2009).

Different approaches have been developed in order to analyze the latent benefits of human iPS cells in animal models of PD. Cai and collaborators employed a commercially available iPS cell line, the IMR90 clone 4, to induce its differentiation towards a mesencephalic dopaminergic lineage in culture. Once they achieved the dopaminergic phenotype, they were transplanted into the striatum of 6-OHDA unilateral lesioned rats. Six weeks after the transplant, they analyzed survival and integration of the transplanted cells within the host brain. They reported that several transplanted cells survived, but some of them expressed nestin and Ki67 which are expressed in dividing cells, such as tumoral cells (Cai et al., 2010). This work however, does not allow conclusions regarding physiological effects, since behavioral outcome was not analyzed.
Later in that year, Hargus et al. employed dopaminergic neurons derived from factor-free iPS cells and transplanted them into the striatum of 6-OHDA unilateral lesioned rats; they later analyzed development, growth, survival and degeneration in an in vivo longitudinal study. They observed a high level of cell survival with no apparent degeneration; they also detected axonal outgrowth in different areas, this can be translated to a soaring capacity of the adult brain to keep axonal guidance instructions that allow the transplanted cells to integrate within the adult brain. In addition, a progressive reduction of motor asymmetry (about 70%) was observed in the transplanted animals over a period of sixteen weeks, 50% of the transplanted DA neurons were identified as SNpc mesencephalic cells and presence of tumoral cells was null (Hargus et al., 2010). However, synaptic function analysis and a longer examination of transplant survival still remain to be done. Besides, the improvement of motor asymmetry was similar to the one reported in other experimental trials that employed different cells, in that aspect, there is no greater advantage of iPS cells over ES cells.

In 2010, reprogramming mice fibroblast directly into a neuronal phenotype directly to a pluripotency stage in vitro was achieved. A combination of 3 genes specifically expressed in neural tissue, Ascl1, Brn2 y Myt1l, was transduced by means of a lentiviral vector. These kind of cells were called induced neurons (iN), since they expressed neural proteins, generate action potentials and form functional synapses; most of these cells express an excitatory phenotype that was later confirmed by cortical neural markers and only a low number of these cells belong to the GABAergic phenotype (Vierbuchen et al., 2010). Yet, it still remains to be determined if iN can be derived from fibroblasts obtained from patients afflicted with a neurodegenerative disease, and which transcription factors must be overexpressed in order to induce different neuronal phenotypes.

Regardless of all of the possibilities that iPS cells might offer, the uncertainty about the latency of tumor formation by being employed in experimental trials still remains. Albeit, better methods to induce reprogramming are currently being developed, but even with viral vector removal, this possibility cannot be totally excluded.

Additionally, recent results demonstrate that production of aberrant methylation sites are originated during the epigenomic reprogramming of iPS cells. Methylation of human iPS cells was observed recurrently at different rates throughout the reprogramming process. Furthermore, the variability of the methylated sites is often seen in iPS cell lines, which indicates that there are some regions more prone to insufficient or aberrant reprogramming. These methylated sites are categorized as “hotspots” for incorrect epigenomic reprogramming, this alteration is not a simple anomaly exclusive of the pluripotent state, it can also be transmitted through iPS cells differentiation (Lister et al., 2011).

So far, iPS cells cannot be employed as a feasible resource for cellular replacement therapy and further work needs to be developed before a clinical trial could take place; however, without a doubt, they possess great potential to be employed as tools for patient-specific disease modeling.

Finally, if we were to choose the type of cell that would bestow the best results after transplantation, taking into consideration the information aforementioned; we believe that the most promising candidate today is the ES cell. In order to provide this personal opinion, we are leaving aside the ethical issues that embrace them, albeit this kind of concerns are of the outmost importance, they are out of the scope of this chapter.
<table>
<thead>
<tr>
<th>Cell Source</th>
<th>In vitro protocol for TH cells production</th>
<th>Recipient</th>
<th>Result</th>
<th>Time period</th>
<th>TH neuron count (after transplant)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse Embryonic Stem Cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mES cells-derived dopamine neurons</td>
<td>ES cultured on PA6 stromal cell</td>
<td>6-OHDA unilateral lesion Mouse</td>
<td>N/A</td>
<td>2 weeks</td>
<td>13,000 (~22% of the original graft)</td>
<td>Kawasaki et al., 2000</td>
</tr>
<tr>
<td>mES cells (no differentiated)</td>
<td>Embryoid body formation stage</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Decrease in 46% of ipsilateral rotation (amphetamine)</td>
<td>9 weeks</td>
<td>NA</td>
<td>Bjorklund et al., 2002</td>
</tr>
<tr>
<td>mES cells-derived dopamine neurons</td>
<td>Genetically-engineered to over express Nurr1</td>
<td>6-OHDA bilateral lesion Rat</td>
<td>Total reversal of ipsilateral rotation (amphetamine)</td>
<td>8 weeks</td>
<td>20,000</td>
<td>Kim et al., 2002</td>
</tr>
<tr>
<td>mES cells-derived dopamine neurons</td>
<td>Nuclear transfer-derived ES (mES). Coculture with stromal cells</td>
<td>6-OHDA unilateral lesion Mouse</td>
<td>Decrease in 80% of ipsilateral rotation (amphetamine)</td>
<td>8 weeks</td>
<td>~22,000 (some reaching 40,000)</td>
<td>Barberi et al., 2003</td>
</tr>
<tr>
<td>mES cells-derived dopamine neurons</td>
<td>Mice transfected with GFP differentiated using 5 stages protocol</td>
<td>6-OHDA unilateral lesion Mouse</td>
<td>Decrease in 50% of ipsilateral rotation (amphetamine)</td>
<td>2 weeks</td>
<td>NA</td>
<td>Nishimura et al., 2003</td>
</tr>
<tr>
<td>mES cells-derived dopamine neurons</td>
<td>Differentiated using 5 stages protocol</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Total reversal of ipsilateral rotation (amphetamine) Starting at 4 weeks</td>
<td>52 weeks</td>
<td>5,000</td>
<td>Rodriguez-Gomez et al., 2007</td>
</tr>
<tr>
<td>mES cells-derived from parkinsonian mice</td>
<td>Nuclear transfer-derived ES (mES). Coculture with stromal cells</td>
<td>6-OHDA unilateral lesion mouse</td>
<td>Total reversal of ipsilateral rotation (amphetamine) Starting at 4 weeks</td>
<td>10 weeks</td>
<td>Avg 8,784 ± 4,293 cells in the group with better results</td>
<td>Tabar et al., 2008</td>
</tr>
<tr>
<td>Mesencephalic DA neurons</td>
<td>Ngn2-GFP mice Pitx3-GFP mice Nestin-GFP mice Sox2-GFP mice</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Total reversal of ipsilateral rotation (amphetamine) Starting at 3 weeks</td>
<td>6 weeks</td>
<td>440</td>
<td>Jönsson et al., 2009</td>
</tr>
<tr>
<td>Non-human primates Embryonic Stem Cells (monkey ES cells)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-human primates ES cells</td>
<td>Neural progenitors expanded as neurospheres. FGF2 addition</td>
<td>MPTP bilateral lesion Cynomolgus monkeys</td>
<td>Behavioral improvement in neurological score</td>
<td>14 weeks</td>
<td>2,100 per side</td>
<td>Takagi et al., 2005</td>
</tr>
<tr>
<td>Human Embryonic Stem cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hES cells</td>
<td>Culture of hES cells enriched with neural progenitors</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Decrease in 45% of ipsilateral rotation (amphetamine)</td>
<td>12 weeks</td>
<td>389 (0.18% of the graft)</td>
<td>Ben-Hur et al., 2004</td>
</tr>
</tbody>
</table>
One of the major difficulties in restoring the motor functionality in PD through transplant therapy is the correct integration of grafted cells into the nigrostriatal pathway; they are required to improve motor symptoms and circumvent the appearance of undesirable side effects, such as dyskinesia (Politis et al., 2010). Grafted cells should not only provide a DA source for the correct modulation of the basal ganglia, but also be able to integrate the nigrostriatal pathway and receive the regulatory input that allows compensatory mechanisms, such as feedback regulation. In addition, the complexity of the adult brain and the lack of a permissive environment to neuronal regeneration make it particularly complicated for even the best non-modified or engineered stem cell to integrate into the basal ganglia motor loop.

Table 3. Stem cell therapy in animal models of PD.

5. Evaluating the functional effectiveness of Stem Cell Therapy for PD

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>In vitro protocol for TH cells production</th>
<th>Recipient</th>
<th>Result</th>
<th>Time period</th>
<th>TH neuron count</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>hES cells-derived dopamine neurons</td>
<td>Culture on PA6 cells (stromal cells)</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>No improvement (amphetamine)</td>
<td>13 weeks</td>
<td>10-50</td>
<td>Brederlau et al., 2006</td>
</tr>
<tr>
<td>hES cells-derived dopamine neurons</td>
<td>Cocultured with human telomerase-immortalized midbrain astrocytes</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Total reversal of ipsilateral rotation (apomorphine)</td>
<td>Since 6 weeks</td>
<td>26,000 per mm³</td>
<td>Roy et al., 2006</td>
</tr>
<tr>
<td>hES cells-derived dopamine neurons</td>
<td>Generation of spherical neural masses (SNMs) (rossetes)</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Decrease in 49.43% of contralateral rotation (apomorphine) Decrease in 58.37% of ipsilateral rotation (amphetamine)</td>
<td>12 weeks</td>
<td>10,700 (2.7% of transplanted cells)</td>
<td>Cho et al. 2008</td>
</tr>
<tr>
<td>iPS clone O9</td>
<td>Reprogrammed fibroblasts (mouse)</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Decrease in 60% of ipsilateral rotation (amphetamine)</td>
<td>8 weeks</td>
<td>29,000</td>
<td>Wernig et al., 2008</td>
</tr>
<tr>
<td>iPS cells IMR90 clone 4</td>
<td>Reprogrammed fibroblasts (human)</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Decline in rotation score</td>
<td>6 weeks</td>
<td>NA</td>
<td>Cai et al., 2010</td>
</tr>
<tr>
<td>iPS cells excisable virus</td>
<td>Human PD (patients)</td>
<td>6-OHDA unilateral lesion Rat</td>
<td>Decrease in 70% of ipsilateral rotation (amphetamine)</td>
<td>16 weeks</td>
<td>122 neurons per mm³</td>
<td>Hargus et al., 2010</td>
</tr>
</tbody>
</table>
Cell grafts might be able to compensate the loss of modulatory inputs from the SNpc by forming local neural circuits within the striatum (Barker et al., 1999; Kreitzer et al., 2008; Lindvall et al., 2004). Ideally, these circuits should be capable to modulate basal ganglia pathways through interaction with D2 and the D1 dopamine receptors, as well as to be subject to feedback regulation in order to prevent variations of DA availability.

In order to evaluate these neural circuits, the need of joined functional and behavioral tests to assess improvement is one of the main objectives. The use of positron emission tomography (PET) coupled to magnetic resonance imaging (MRI) techniques has diminished the gap between behavioral and functional data.

5.1 MRI assessments

Conventional MRI reveals brain structural changes as reductions in volume (atrophy) and alterations in water-proton relaxation T1 and T2 signals. Water normally flows along neural tracts in the brain. Diffusion-weighted or diffusion tensor MRI can be used to quantify loss of anisotropy (directionality) or increase in amplitude of water diffusion in order to demonstrate disruption of neural tracts.

Conventional T1 and T2 weighted MRI show normal nigral structure in idiopathic PD and therefore is not diagnostically helpful. Volumetric T1 weighted MRI studies have also failed to detect a reduction in nigral volume in PD, possibly because of difficulties in accurately defining the border of the SNpc (Geng et al., 2006) and thus it would be the most appropriate technique for evaluating the survival of dopamine-derived transplants.

MRI inversion recovery sequences can be designed to suppress either gray or white matter signal. Segmented inversion recovery ratio imaging generates ratio images of gray matter and white matter suppressed signal at a voxel level. With the segmented inversion recovery ratio approach, PD patients were reported to show a gradient of altered nigral signal that was absent in healthy controls (Hutchinson & Raff, 2000). Although the use of gray matter and white matter suppressing inversion recovery sequences can detect changes in nigral structures in PD, it is a complicated approach to implement and currently not sensitive enough to be of diagnostic value in transplant therapy.

Regardless of the limitations previously mentioned, MRI is a non-invasive, real-time cellular imaging modality that has no radiation, and for these reasons, several attempts have been made in order to take advantage of these qualities. New MRI techniques currently employ magnetic labeling using superparamagnetic iron oxide particles (SPIOs) coupled to stem cells transplanted in the rat striatum with the purpose of assessing the survival time of transplanted tissue (Berman et al., 2011; Obenaus et al., 2011). Nevertheless, this innovative technique is only useful for quantifying cell survival and does not provide data to evaluate transplanted cell function.

5.2 PET assessments

Terminal dopa decarboxylase activity and dopamine turnover can be assessed using radioactive markers such as 6-[18F]-fluoro-L-DOPA (18F-DOPA), which could be helpful for the evaluation of PD progression in vivo (Brooks et al., 2008) and graft survival in transplanted PD patients (Mendez et al., 2002; Sawle et al., 1992). Early hemiparkinsonian patients show a bilateral reduced putamen dopaminergic terminal function. Clinical parkinsonism occurs when PD patients have lost 40%–50% of posterior putamen-dopamine terminal function (Morrish et al., 1995). Levels of 18F-dopa uptake in the putamen and DAT
binding correlate inversely with bradykinesia and rigidity of PD patients, and could be valuable for estimation of cell therapy functional recovery. However, this type of analysis has limitations; \(^{18}\)F-DOPA is not specific for evaluation of DA re-uptake in small animal PET scanning, which is an essential feature for the assessment of emergent cell therapy in animal models of PD. Other radiotracers of DAT binding, such as \(^{11}\)C-(+)-α-dihydrotetrazenzine (\(^{11}\)C-(+DTBZ)), have better specificity and spatial resolution when evaluating animal models of PD (Collantes et al., 2008) and could be used to examine the survival of DA neurons. Still, in order to correctly evaluate DA release, it is necessary to use radioactively labeled dopamine receptor antagonists, such as \(^{11}\)C-raclopride, which can be displaced from DA receptors by DA released from nerve terminals located in situ or exposed to amphetamine (Piccini et al., 2000). \(^{11}\)C-raclopride PET is able to indirectly detect synaptic dopamine fluxes by monitoring changes in striatal D2 receptor availability (Laruelle, 2000). The higher the extracellular dopamine level, the lower the dopamine D2 site availability to the tracer. Animal microdialysis studies suggest that a 25% fall in putamen \(^{11}\)C-raclopride uptake after amphetamine equates to a 10-fold rise in synaptic dopamine levels (Breier et al., 1997). In PD patients, when given L-DOPA treatment, they show a fall in striatal 11C-raclopride binding; the response of bradykinesia and rigidity to L-DOPA in PD correlates with the resulting increase in striatal dopamine levels detected with 11C-raclopride PET (Pavese et al., 2006). By means of \(^{18}\)F-DOPA and \(^{11}\)C-raclopride PET scans, it has been shown that grafted (human embryonic mesencephalic and fetal dopaminergic tissue) cells remain active and form connections with neurons residing in the host striatum when transplanted in human brains (Mendez et al., 2008; Piccini et al., 1999; Spencer et al., 1992). The major drawback is that the spatial resolution achieved with the current available PET scanners (2 × 10\(^{-3}\) m) is insufficient to evaluate single cell events, which can only be resolved with 100-fold higher resolutions (10–20 \(\mu\)m).

So far, the information already described shows that current MRI and PET analysis have some limitations for a correct cell graft evaluation of survival and integration within the host, specifically at the cellular level.

### 5.3 Microcircuits on brain slice preparations: the need for functional studies

It is clear that better protocols for cell replacement therapy would benefit from a better understanding of normal basal ganglia circuit functions. One of us (Bargas’ group) designed a brain slice preparation in which individual cell activity can be observed and evaluated, using calcium-imaging techniques (Carrillo-Reid et al., 2008). When corticostriatal slices are loaded with a calcium-sensitive fluorescent dye, it is possible to analyze single-cell activity in a widespread area of the striatum. Additionally, it is possible to examine the activity of cell clusters that have an associated firing pattern (neural ensembles) and represent functional microcircuits within the striatum (Carrillo-Reid, 2008, 2009). Different cells within the striatum belong to different ensembles, and there are some cells that belong to most of the ensembles present in a given slice. These cells serve as central pattern generators (CPGs), which are considered to represent memory traces in the nervous system (Carrillo-Reid, 2008, 2009; Grillner, 2005, 2006). CPG activity within the striatum might be related to the encoding of motor programs that are activated secondary to cortical stimulation. In this regard, it is worth of notice that unstimulated corticostriatal slices mostly show silent cells, with some uncoordinated activity.
Ensemble activity is triggered by stimulation of cortical fibers or after exposure to glutamatergic agonist NMDA. These results suggest the existence of intrinsic microcircuits in the striatum, which could be the actual modulators of motor activity in this brain area. Thus, it would be necessary to determine if the segregated pathways represented by neurons expressing either the D1 or the D2 dopaminergic receptors, belong to distinct activity ensembles; this would be of major interest, since deregulation of the D1 receptor pathway has been proposed to be responsible for the dyskinesias observed in PD patients after prolonged exposure to L-DOPA (Dupre et al., 2008; Santini et al., 2008; Taylor et al., 2005). Of particular interest would be to demonstrate if there is a difference between ensembles in the intact brain and those in the affected striatum of PD patients. We propose that the use of this brain slice preparation will allow us to study if transplanted dopaminergic cells can integrate into local microcircuits within the striatum, and if formation of new modulatory connections could compensate for the loss of the dopaminergic input from the SNpc and reestablish control of motor function.

6. Conclusion

This chapter highlights that although almost three decades have passed since the Backlund-Lindvall and Drucker-Colin et al. work was published, transplant therapy for PD is still retained as a potential clinical approach; stem cell research is currently providing a more adequate source of cells. So far, the large body of evidence appears to validate the use of manipulated stem cells in the coming years. Although stem cell therapy shows promise, it is still in development, and even with a wide range of methods, currently there is no ideal scenario for clinical transplantation and perhaps it will not arrive in the near future. ES cell therapy still remains elusive and is burdened with social and logistical concerns. In contrast, iPS generation circumvents some previous limitations, since it does not require embryonic material. iPS cell technology might have a significant impact on regenerative medicine in the near future.

In order for cell therapy to become a viable option of treatment, a pure source of dopaminergic neurons is essential. However, the poor survival of transplanted differentiated cells limits transplant therapy; ergo a pure population would not be the best option, since cell survival decreases after transplantation. Additionally, the combination with other cell populations could also be necessary for a successful transplant, given the fact that primary fetal cells that provide behavioral improvement are only ~20% of the total implanted cells. Finally, it should be particularly noted, that aside of a couple of studies, all report incomplete motor improvements. The search for the basis of this result is today still in its embryonic stage. As a final point, research with better surgical procedures that provides controlled assessments consequently will lead to a more clear understanding of the phenomena involved grafting in PD.

In sum, despite several failures, we believe that cell replacement therapy has a viable future.

7. Acknowledgments

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8. References


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Based on our current understanding of cell biology and strong supporting evidence from previous experiences, different types of human stem cell populations are capable of undergoing differentiation or trans-differentiation into functionally and biologically active cells for use in therapeutic purposes. So far, progress regarding the use of both in vitro and in vivo regenerative medicine models already offers hope for the application of different types of stem cells as a powerful new therapeutic option to treat different diseases that were previously considered to be untreatable. Remarkable achievements in cell biology resulting in the isolation and characterization of various stem cells and progenitor cells has increased the expectation for the development of a new approach to the treatment of genetic and developmental human diseases. Due to the fact that currently stem cells and umbilical cord banks are so strictly defined and available, it seems that this mission is investigational more practical than in the past. On the other hand, studies performed on stem cells, targeting their conversion into functionally mature tissue, are not necessarily seeking to result in the clinical application of the differentiated cells; In fact, still one of the important goals of these studies is to get acquainted with the natural process of development of mature cells from their immature progenitors during the embryonic period onwards, which can produce valuable results as knowledge of the developmental processes during embryogenesis. For example, the cellular and molecular mechanisms leading to mature and adult cells developmental abnormalities are relatively unknown. This lack of understanding stems from the lack of a good model system to study cell development and differentiation. Hence, the knowledge reached through these studies can prove to be a breakthrough in preventing developmental disorders. Meanwhile, many researchers conduct these studies to understand the molecular and cellular basis of cancer development. The fact that cancer is one of the leading causes of death throughout the world, highlights the importance of these researches in the fields of biology and medicine.

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