Dopamine cell transplantation in Parkinson’s disease: challenge and perspective

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Background: Functional imaging provides a valuable adjunct to clinical evaluation for assessing the efficacy of cell-based restorative therapies in Parkinson's disease (PD).

Sources of data: In this article, we review the latest advances on the use of positron emission tomography (PET) imaging in evaluating the surgical outcome of embryonic dopamine (DA) cell transplantation in PD patients.

Areas of agreement: These studies suggest long-term cell survival and clinical benefit following striatal transplantation of fetal nigral tissue in PD patients and in models of experimental parkinsonism.

Areas of controversy: Adverse events subsequent to transplantation have also been noted and attributed to a variety of causes.

Growing points: Optimal outcomes of DA cell transplantation therapies are dependent on tissue composition and phenotype of DA neurons in the graft.

Areas timely for developing research: Given continued progress in DA neuron production from stem cells in recent years, transplantation of neural stem cells may be the next to enter clinical trials in patients.

Conclusion: The existing data from studies of embryonic DA transplantation for advanced PD have provided valuable insights for the design of new cell-based therapies for the treatment of this and related neurodegenerative disorders.

Keywords: cell transplantation/Parkinson’s disease/regenerative medicine/emission tomography

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Introduction

Parkinson’s disease (PD) is a common movement disorder resulting primarily from progressive degeneration of nigrostriatal dopaminergic (DA) neurons. The cardinal symptoms of PD are linked to the attrition of DA afferents to the posterior putamen and consequent impairment in the functioning of cortico-striato-pallido-thalamo-cortical (CSPTC) motor circuits. Pharmacologic repletion of brain dopamine concentrations with oral levodopa preparations and/or dopamine agonist drugs is clinically effective for the treatment of PD motor manifestations, particularly at the early stages of the illness. This DA replacement therapy results in a non-physiological release of dopamine in the brain. Nonetheless, chronic treatment is often associated with the progressive development of response fluctuations and abnormal involuntary movements known as drug-induced dyskinesias. Over the past decade, a number of stereotaxic surgical approaches have been introduced to correct the CSPTC motor circuit abnormalities that are characteristic of PD. This is based on ablative lesioning or deep brain stimulation (DBS) on subcortical targets. While these neurosurgical interventions offer viable clinical benefits for patients, their long-term efficacy is still to be established. An earlier strategy was designed to address the issue more fundamentally by attempting to reinnervate the dopaminergically depleted striatum by implanting healthy DA neurons into the posterior putamen target region. Fetal cell transplantation offers an opportunity to actually replace synapses and restore physiological dopamine transmission in the brain. This has the potential to deliver superior treatment efficacy in PD patients that is sustained over the long term.

Beginning in the early 1990’s, a variety of clinical studies has been conducted to evaluate the safety and efficacy of fetal DA cell transplantation into the striatum of advanced PD patients. The strategy motivating this intervention has been described in detail elsewhere. Briefly, grafts derived from human embryonic mesencephalic tissue are implanted into the striatum of patients with PD. The tissue is harvested 6–9 weeks post-conception and the transplantation typically requires one to four donors per side. Immunotherapy may be administered for a period of time to promote graft survival and to suppress immune reactions from the host tissue. Clinical outcome is assessed by changes in standardized motor ratings taken with the patients off anti-parkinsonian medications for at least 12 hours overnight.

Functional brain imaging with positron emission tomography (PET) can provide sensitive in vivo biomarkers of dopaminergic dysfunction in PD patients. This is achieved by measuring DA-specific presynaptic
and postsynaptic indices with appropriate radiotracers. In particular, \([^{18}\text{F}]\)-fluorodopa (FDOPA) PET measures the rate of dopamine decarboxylation and is therefore more sensitive in gauging graft viability than other imaging methods used for assessing the integrity of nigrostriatal terminals (e.g. dopamine transporter-binding radioligands)\(^6\) and dopamine release (e.g. \([^{11}\text{C}]\)-raclopride)\(^7\) following transplantation. In addition, the recovery of cortical function may be evaluated indirectly by mapping DA-regulated changes in cerebral blood flow or metabolism in the striatum and associated brain circuitry.\(^8\) Thus, FDOPA PET method is commonly used to provide an index of the recovery of the nigrostriatal DA system after neural transplantation. If successful, DA cell transplantation should increase and sustain the levels of this parameter postoperatively.

In an earlier review,\(^5\) we summarized the results of the first double-blind, placebo-controlled trial over 1 year and contrasted these findings with those from open-label trials in small PD cohorts and in experimental disease models. Synaptic contacts between transplanted fetal DA cells and host neurons have been described consistently in several reports in PD patients. Indeed, recent studies in rodent models have demonstrated that it is the presence of DA terminal transmission and not dopamine levels \textit{per se} that restores function in transplantation paradigms,\(^9\) and exacerbation of ultrastructural abnormalities in these synaptic contacts may induce graft-induced aberrant behaviors.\(^10\) Subsequent studies demonstrated how intraputaminal embryonic DA cell grafts exhibit long-term survival, provide extensive striatal reinnervation, and improve motor function. There has also been a deeper understanding of the mechanisms underlying graft-related side effects including dyskinesias. At the same time, researchers have begun preclinical testing of new cell-based treatments, including the implantation of neural stem cells (NSCs). This review will summarize the lessons learned from clinical trials of embryonic DA cell grafts in PD patients. We will also discuss the implications of recent developments in stem cell research for future transplantation trials in PD.

**Fetal cell transplantation**

Most early clinical trials on DA cell replacement therapy have used small numbers of patients in an open-label experimental design. This strategy is important for methodological development, but can be statistically flawed by uncontrolled placebo effects as well as investigator bias. That said, this early work set the stage for two larger double-blind, placebo-controlled surgical trials of human embryonic DA cell transplantation in PD.
In the first study, 40 patients with severe PD (age $57 \pm 10$ years) were randomly assigned to placebo surgery or transplantation in the bilateral putamen of cultured tissue from two embryos per side without immunosuppression. After the blinded follow-up in 39 surviving patients at 1 year, there was a significant improvement in Unified Parkinson’s Disease Rating Scale (UPDRS) motor scores in transplantation patients as compared with placebo-surgery patients. This improvement was present in the younger patients (age $<60$ years) but not in the older patients (age $>60$ years). In addition, no changes were detected in cognitive function or in personality traits in both transplant and sham-operated groups. Hence, the implantation of embryonic DA neurons was regarded as safe in PD patients, without affecting cognition or personality.

In an initial study, FDOPA PET imaging was performed preoperatively and repeated at 1 year post-surgery under blinded conditions, and changes in putamen radiotracer uptake were compared in the transplant group relative to the sham-operated subjects. Increases in FDOPA uptake were evident in the transplant group irrespective of subject age. Post-transplantation increases in PET signal were found to correlate with clinical improvement in the young ($\leq 60$ years) but not in the older (>60 years) transplant recipients. These findings suggested that age does not affect graft viability measured by post-transplantation increases in FDOPA uptake during the first postoperative year. However, host age may alter the time course of the downstream functional changes that are necessary for clinical benefit to occur.

In the second double-blind study, 34 PD patients (age $59 \pm 8$ years) underwent embryonic DA cell transplantation in the bilateral putamen with one or four donors per side or placebo surgery, along with immunosuppressive therapy over 6 months. Although there was no overall clinical effect, PET data showed significant increases in putaminal FDOPA uptake in both one- and four-donor transplant groups at the end of the 2-year follow-up period. This study did not observe a significant treatment effect in patients $<60$ years old, but reported clinical benefits of transplants in less severely affected patients (UPDRS motor $\leq 49$; $P < 0.01$), which persisted up to 2-year post-surgery. Of note, relative to placebo-treated subjects, the transplant recipients were found to improve ($P < 0.05$) during the period of immunosuppression, but deteriorated afterward. By contrast, another study showed no evidence of clinical deterioration and compromised graft survival in six PD patients after the withdrawal of immunosuppression at 29 months post-transplantation. In both double-blind clinical trials, the magnitude of improvement in clinical and PET measures corresponded to that observed in open-label studies and no significant change was observed in the placebo-operated groups. Furthermore, post-mortem
studies revealed histopathological evidence of graft survival and dopaminergic reinnervation in several patients who were found in life to have increased striatal FDOPA uptake and who subsequently died of unrelated causes.\(^2,3\)

In a recent study, we explored the long-term outcome of embryonic cell transplantation in the original placebo-controlled cohort after the blind was lifted.\(^1,4\) Fourteen of the 20 placebo-operated patients in our original study received transplants after completing the blinded phase of the trial (i.e. were ‘crossed over’), resulting in a total of 33 graft recipients. UPDRS motor ratings and FDOPA PET scans (Fig. 1) were obtained from all 33 subjects at baseline and at 2 years after transplantation and 15 of the subjects were again evaluated clinically and with FDOPA PET at 4 years.

Table 1 presented all clinical data and striatal FDOPA uptake values from the transplant recipients with long-term follow-up. Relative to the preoperative baseline, UPDRS motor scores improved significantly \((P < 0.01)\) by 21–43% at the first and second post-transplantation time points (1 and 2 years), and by 25% at the third (4 years) time point (Fig. 2). Clinical improvement peaked at 2 years post-surgery but was found to have declined slightly at 4 years. Interestingly, clinical improvement was relatively greater for the young patients \((<60\text{ years at the time of implantation})\), as well as for the male transplant recipients at 1 year. However, these age/gender differences were not present in the long term. Concurrently, putamen FDOPA uptake increased significantly \((P < 0.05)\) by 26, 32 and 46% at the three post-transplantation time points (Table 1; Fig. 3). These changes were not influenced by subject age and gender, and exhibited a significant correlation \((P < 0.05)\) with concurrent measures of clinical outcome. As expected, FDOPA uptake did not change over the same period of time in the non-grafted caudate nucleus.

![Fig. 1 FDOPA PET images of one representative patient with advanced Parkinson’s disease scanned at baseline (PRE) and at 1 (POST-1Y), 2 (POST-2Y) and 4 (POST-4Y) years after embryonic dopamine cell transplantation in the bilateral putamen. All postoperative scans show gradual increases in FDOPA uptake particularly in the posterior putamen (white arrows) where dopamine loss is the largest preoperatively.](image-url)
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<th></th>
<th>PRE</th>
<th>POST-1Y</th>
<th>POST-2Y</th>
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<tr>
<td><strong>UPDRS motor score</strong></td>
<td>37.5 ± 3.4</td>
<td>29.6 ± 4.4</td>
<td>21.5 ± 2.7</td>
<td>28.1 ± 2.7</td>
<td>-20.9 ± 5.0**</td>
<td>-42.7 ± 9.2**</td>
<td>-24.9 ± 4.7**</td>
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<td><strong>Putamen FDOPA uptake</strong></td>
<td>0.51 ± 0.03</td>
<td>0.64 ± 0.04</td>
<td>0.67 ± 0.04</td>
<td>0.74 ± 0.05</td>
<td>25.7 ± 3.0*</td>
<td>31.6 ± 4.0**</td>
<td>45.7 ± 6.1**</td>
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<td><strong>Caudate FDOPA uptake</strong></td>
<td>0.78 ± 0.03</td>
<td>0.74 ± 0.02</td>
<td>0.77 ± 0.03</td>
<td>0.77 ± 0.03</td>
<td>-5.2 ± 0.4</td>
<td>-2.1 ± 0.2</td>
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Data (mean ± standard error) represent off-state UPDRS motor ratings and striatal FDOPA uptake from 15 transplant recipients measured at least 12 h off anti-parkinsonian medication at baseline (PRE) and at 1 (POST-1Y), 2 (POST-2Y) and 4 (POST-4Y) years post-transplantation, respectively. Striatal FDOPA uptake is defined as \((\text{region-occipital})/\text{occipital}\) at 95 min after tracer injection. The lower motor ratings reflect less severe parkinsonism; the higher uptake values reflect better dopaminergic function. \(\triangle = (\text{postoperative} - \text{baseline})/\text{baseline} \times 100\%.\) The more negative values in UPDRS change reflect greater improvement in parkinsonian signs; the more positive values in FDOPA uptake change reflect greater improvement in dopaminergic function.

\(*P < 0.05, **P < 0.01, ^*P < 0.005, ^{++}P < 0.0005\) compared with the preoperative baseline (paired Student’s t-tests).
Fig. 2 Off-state UPDRS motor ratings (mean ± SE) at baseline (PRE) and at 1 (POST-1Y), 2 (POST-2Y), and 4 (POST-4Y) years after embryonic dopamine cell transplantation in the 15 participants who were evaluated at all four time points. A significant treatment effect over time \((P < 0.001)\) was evident in these subjects (A, B). The graph on the left side (A) was modified from Fig. 1 in reference Ma et al.\(^{14}\) The operative change is defined as \((\text{Postoperative–Baseline}) / \text{Baseline} \times 100\%\). The asterisks represent \(P\)-values with respect to preoperative baseline. **\(P < 0.01\), +\(P < 0.0005\).

Fig. 3 Striatal FDOPA uptake (mean ± SE) measured at baseline (PRE) and at 1 (POST-1Y), 2 (POST-2Y), and 4 (POST-4Y) years after embryonic dopamine cell transplantation in the 15 transplant recipients scanned at all four time points. FDOPA uptake in the putamen increased significantly \((P < 0.001)\) following implantation (A, B), whereas there was no change in the non-grafted caudate (C, D). The graphs on the left side (A, C) were modified from Fig. 1 in reference Ma et al.\(^{15}\) The data in the graphs were generated for the putamen and caudate from pre-defined anatomical volumes of interest and plotted as percentages of the corresponding normal mean values. The dotted lines represent the lower limit of striatal FDOPA uptake (mean – 2 SD) in the controls. The asterisks represent \(P\)-values with respect to preoperative baseline. *\(P < 0.05\), **\(P < 0.01\), +\(P < 0.005\).
To localize the effects of DA grafts at the voxel level, we examined the time course of FDOPA uptake in the brains of the transplant recipients using a parametric mapping approach (Fig. 4). Compared to the preoperative baseline scans, the signal increases post-transplantation were localized bilaterally ($P < 0.001$) to the posterior putamen implantation site. The functional imaging increases in this region are likely responsible for the continued clinical improvement observed at the 2-year time point. Interestingly, concurrent declines in FDOPA uptake were observed in the non-engrafted caudate and rostroventral putamen, indicating ongoing degeneration of the nigral DA projections to these regions in the period following implantation. Of note, the clinical improvement seen at 1 and 2 years after transplantation correlated positively with preoperative PET signal in this region. These findings

![Fig. 4 Changes in striatal FDOPA uptake from baseline (PRE) at 1 (POST-1Y), 2 (POST-2Y), and 4 (POST-4Y) years after embryonic dopamine cell transplantation in the 15 transplant recipients who were scanned at all four time points. Transplantation resulted in ($P < 0.001$) increased FDOPA uptake in the posterior putamen (A) as well as concurrent bilateral reductions in the non-grafted caudate and ventrorostral putamen (C) using brain mapping analysis on a voxel-to-voxel basis. Subsequent regional analysis revealed progressive increases in FDOPA uptake in the posterior putamen (B) and decreases in the ventrorostral putamen (D). The graphs on the right side (B and D) were modified from Fig. 3 in reference Ma et al. The regions with significant change in FDOPA uptake were superimposed over a standard MRI brain template. The data in the graphs were generated from spherical volumes of interest (4 mm radius) centered at the peak coordinates from the voxel-based analysis.]
accord with those of an earlier imaging study,⁷ and suggest that retention of PET signal in non-motor regions of the striatum may be a useful preoperative marker of the clinical response to engraftment. That PET signal in these regions was found to decline over this time period points to the possibility of a discrete ‘time window’ for this and related cell-based therapies for advanced PD.

The data from the 4-year time point were found to be highly informative. Firstly, clinical improvement, increased putamen FDOPA uptake, and clinical-PET correlations proved to be robust despite the substantial loss to follow-up of the transplant recipients after the 2-year time point. Secondly, the data allowed us to assess the long-term impact of the five transplant recipients who had developed graft-induced dyskinesia² (see below). Interestingly, similar clinical outcomes and PET changes were seen across the entire cohort whether or not these dyskinesia subjects were included in the analysis. These findings indicate that clinical response to transplantation, as well as graft viability, are not driven by individuals exhibiting this side effect.

These long-term clinical and PET findings accord with earlier reported observations based upon the blind-phase data, as well as open-label studies conducted over an analogous period of observation. The significant relationship between graft-mediated changes in clinical ratings and FDOPA uptake was also reported in another open-label study.⁶ These observations support the use of FDOPA PET in the evaluation of DA cell-based therapies. In particular, the robust clinical outcome and FDOPA uptake increases were in line with the previous reports that immunosuppression permitted a reduction in the amount of grafted tissue, but did not induce a greater recovery in terms of symptomatic relief and graft survival.⁷,¹⁵ In this vein, it is not known why further clinical and imaging improvements were not seen between 2 and 4 years after implantation (Figs 2 and 4B). It has been suggested that α-synuclein pathology can develop in the grafts.¹⁶,¹⁷ That said, in our cohort only 5 years had passed after transplantation as opposed to more than 10 years in the reported pathologic studies. Moreover, pathology-free long-term graft survival has been demonstrated in five autopsied subjects examined 9–14 years after DA cell transplantation.¹⁸ This is in agreement with observations from other studies with long-term follow-up showing improved motor function and increased FDOPA uptake for up to more than 10 years post-transplantation.¹⁹,²⁰

**Potential complications following transplantation**

Complications related to the intrastriatal transplantation of human embryonic mesencephalic tissue have generally been reported to be...
mild and transient as a result of functional neurosurgery. Nonetheless, a number of graft recipients experienced the gradual onset of abnormal dyskinetic movements post-transplantation occurring in the absence of or with only minimal dopaminergic medication. Specifically, persistent dyskinesias have been seen in the off-medication state in 5 of the 33 patients (15%) who received bilateral putamen DA grafts in the first randomized placebo-controlled trial. In these subjects, the graft-induced dyskinesias (GID) emerged between the first and second postoperative years following substantial clinical improvement in the first post-transplantation year. Of note, no additional patients in this transplant cohort developed GID when followed clinically over the long term. In a subsequent imaging study, caudate and putamen FDOPA uptake values in the five dyskinesia patients were compared with those from 12 clinically matched transplant recipients who did not develop this complication. We found that putamen FDOPA uptake was significantly higher in the dyskinetic graft recipients at both 1 and 2 years following transplant surgery ($P < 0.005$). Voxel-wise mapping revealed that the increases in PET signal were predominantly localized to two zones within the left putamen: the posterodorsal area in which a prominent reduction in FDOPA uptake was present at baseline; the posteroverentral area in which preoperative dopaminergic input was relatively preserved. Postoperative FDOPA uptake in either region did not reach supernormal levels over the 2-year period. Localized increases in graft-related PET signal have also been observed in experimental animal models of GID. In aggregate, the findings suggest that this complication of DA cell implantation is associated with unbalanced increases in regional dopaminergic function within the striatum.

Dyskinesia was also found to occur in 13 of 23 transplant patients (56%) enrolled in the second double-blind clinical trial of embryonic DA cell implantation for PD. However, in this study, no differences were observed in regional or global striatal FDOPA uptake in the GID patients relative to uncomplicated graft recipients. Of note, this adverse effect occurred approximately 3 months after the cessation of immunosuppression. The large fraction of dyskinetic patients in this trial has been attributed to a possible inflammatory response in the grafts, as suggested by animal studies. Indeed, another study disclosed worsening dyskinesia in transplant recipients following the withdrawal of long-term immunosuppression over a mean period of 29 months after the last transplantation. This study included eight patients from the open-label retrospective study in which moderate off-state dyskinesias were reported in 6 of 14 transplanted patients (43%) who were followed long term. Nonetheless, dyskinesia severity was not correlated with the magnitude of graft-derived DA innervation or dopamine release in the grafted putamen.
Although there appears to be a link between suboptimal recovery of presynaptic function and GID, the exact mechanism of this phenomenon remains unknown. Dyskinesias are likely to also be influenced by chronic exposure to l-dopa preoperatively, as well as graft–host interactions involving postsynaptic DA receptors or other non-DA neurons. Indeed, GID might be related to increased serotonergic innervation by the grafts as reported recently in two PD patients. PET imaging studies are likely to offer further information concerning the pathophysiology of GID.

**New development and prospective**

Despite the inherent appeal of neural transplantation as a restorative approach to the treatment of PD, a number of challenges remain (see recent reviews). Firstly, there is a need to develop sustainable sources of DA tissue, particularly of stem cell origin. Research is also needed to determine whether adjunctive agents such as neurotrophins can be used to optimize graft viability and integration of the transplant into the host brain. Lastly, there is a need to develop improved imaging techniques for more accurate and objective assessment of transplantation outcome.

**Alternative sources of dopamine tissue**

Cell replacement therapy generally requires large amounts of human embryonic mesencephalic tissue to achieve sustainable therapeutic effects. Adequate supplies of human tissue are not readily available because of ethical and practical considerations, and alternative sources of viable DA cells are being sought. For example, human retinal pigment epithelial (hRPE) cells can be easily harvested from donor eyes, grown in culture and implanted in the brain after being attached to gelatin microcarriers. hRPE cells naturally produce l-dopa as a neuromelanin precursor. The long-term safety of striatal hRPE implants has been reported in parkinsonian primates, with appreciable increases in striatal FDOPA uptake following the implantation of hRPE cells. PET with \([^{18}\text{F}]-\text{FDG}\) has also been used to investigate the system-level effects of hRPE cell implantation in a primate model. Eight macaques with bilateral 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism were evaluated before and after unilateral striatal implantation of either hRPE cells \((n = 5)\) or placebo \((n = 3)\). There was evidence of significant clinical improvement as well as graft-mediated reductions in the expression of an abnormal parkinsonism-related metabolic covariance pattern,
homologous to the PD-related spatial covariance pattern, which characterizes metabolic substrates in patients with PD. This study supports the use of FDG PET for assessing metabolic effects of cell transplantation in the human trial of PD.

In the first open-label pilot study, six young patients with PD (mean age 52.2 years) were investigated without immunosuppression before and 2 years after unilateral implantation of cultured hRPE cells. Clinical benefit was observed in all patients, and off-state dyskinesias were not evident during the same period of follow-up. Despite this promising outcome, a subsequent randomized placebo-controlled trial of hRPE cell implantation for PD did not meet its primary end point.

Production of dopamine neurons by NSCs

NSCs may provide a virtually unlimited source of self-renewing progenitors for transplantation. The potential applications and technical challenges of this approach have been critically reviewed. It has been reported that transplanting differentiated monkey embryonic NSCs into the monkey putamen leads to their proliferation into fully functional DA neurons. Implantation of these DA neurons caused sustained improvement of MPTP-induced motor symptoms that was significant over a 10–14-week follow-up period compared with a sham group. An increase in FDOPA uptake in the grafted putamen was observed at 14 weeks, along with the presence of a large number of surviving DA neurons in the graft at post-mortem. Of note, none of the transplanted animals developed graft-induced dyskinesia.

DA neurons can also be cultivated from human embryonic cells. Proliferation into fully differentiated DA neurons has been observed following the implantation of undifferentiated human NSCs into MPTP-lesioned monkeys. The grafts improved motor function at 2 months post-surgery; MPTP-induced changes in the size and number of host cells were substantially improved at 4 and 7 months. Induced pluripotent stem cells (iPSCs) are currently being developed to create potential sources of patient-specific donor cells for autologous transplantation. This technique is based on the reprogramming of adult somatic cells (e.g. fibroblasts) into pluripotent stem cells. Importantly, iPSCs have the potential to derive DA cell types specific to the transplant recipient, limit the host’s immune response and negate the requirement for immunosuppression. Hence, cell replacement therapy using iPSCs can avoid immunological-related complications associated with the use of embryonic stem cells.

Adult human NSCs may ultimately prove useful for transplantation in the treatment of PD. In a recent study, NSCs isolated from
cortical–subcortical brain tissues were allowed to expand in vitro for several months. 40 Nine months after harvesting, cell suspensions composed of differentiated dopaminergic and GABAergic neurons that were injected unilaterally into the putamen of a young patient with advanced PD. Clinical UPDRS off-state motor ratings improved by 83% between 1 and 3 years following surgery, but began to deteriorate during the fourth post-transplantation year. The clinical ratings returned to baseline by year 5. There was a 56 and 33% increase in FDOPA uptake in the implanted left putamen at 3 and 12 months. The absence of further increase in FDOPA uptake was noted, reflecting reduced survival of grafted DA neurons, continued loss of host DA neurons or both. While these observations are limited to a single case study, the data suggest that the surgery is likely to be safe. It remains uncertain whether the observed motor benefit was attributable to the combination of GABAergic and dopaminergic cells in the graft.

Use of nerve growth factors

Neurotrophins such as glial cell line-derived neurotrophic factor (GDNF) are known to have neuroprotective effects on DA neurons. Small, open-label trials have suggested that GDNF can be effective in improving the survival and functional activity of mesencephalic grafts. 41 That said, it is not clear whether GDNF is effective on its own as a therapeutic agent for PD. For example, a stable improvement (>57%) in motor function was reported in an open-label study of five PD patients who underwent continuous intraputamenal infusion of GDNF over 1–2 years. 42 In a subsequent randomized, controlled trial in 34 patients with moderate-to-severe PD, clinical improvement was not significant at 6 months despite a 32% increase in posterior putamen FDOPA uptake in 22 patients with usable PET images. 43 In this regard, a recent study reported the regeneration of the DA system in parkinsonian monkeys after convection-enhanced delivery of adeno-associated virus-GDNF vectors into the putamen. 44 The investigators reported recovery of striatal dopamine levels and regrowth of nigrostriatal fibers, and progressive amelioration of functional deficits over a follow-up period of 2 years. The ultimate role of growth factors, alone or in conjunction with cell-based therapies, in the treatment of PD will be determined in future studies.

Impact of graft composition

It is known that grafts derived from fetal ventral mesencephalon (VM) or from embryonic stem cells contain multiple cell types of
dopaminergic neurons and non-DA neuron such as serotonergic and GABAergic neurons. While some non-DA cells could be conductive to optimal synaptic integrations between the graft and host tissue, others could have a negative effect on this process. Recently, cell-sorting strategies have been developed to eliminate select non-DA neurons from embryonic stem cell-derived dopamine neurons in a rat study, and given the strong evidence for a serotonergic component to GIDs, such a strategy of purification may eliminate this side effect and improve functional outcomes of cell replacement therapy in PD.

It is also of importance to transplant the correct phenotype of dopamine neurons. Histological studies have revealed that dopamine neurons consist of a number of subtypes located predominantly in the retrorubral field (A8), the dorsal and lateral parts of the substantia nigra pars compacta (A9) and the ventral tegmental area (A10). Indeed, it has been reported that the presence of A9 dopamine neurons in fetal VM grafts delivers a favorable clinical outcome in patients, and A9-deficient grafts do not provide motor improvement and only minimally reinnervate the striatum in a rat model of PD. The use of incorrect phenotype of DA neurons may be the reason behind the limited recovery following hRPE cell implantation in a randomized placebo-controlled trial. This is a critical issue to consider not only for fetal cell transplantation, but also for developing future stem cell-derived therapies.

**Improvement in transplantation strategy**

Current cell transplantation surgery in PD has been chiefly on reinnervating the striatum. Evidence from preclinical data suggests that simultaneous intrastriatal and intranigral grafts may produce a more complete functional recovery. An early study evaluated a number of patients who received implants of fetal DA cell suspension in putamen and substantia nigra bilaterally. Improvement was noted in clinical ratings along with an increase in FDOPA uptake in the putamen and substantia nigra at 1 year following implantation. The merits of this strategy will need to be assessed objectively by blinded comparison with conventional protocols.

It has been suggested that the time course of the clinical response to striatal DA cell transplantation for PD is age dependent, and may vary across subjects according to baseline clinical status and the spatial extent of denervation noted preoperatively. This calls for a surgical strategy that is customized to the individual patient. FDOPA PET (and related imaging methods for assessing the integrity of nigrostriatal terminals) can provide an objective criterion for patient selection and
optimization of transplantation target sites. Moreover, newer surgical
techniques designed to produce more homogeneous reinnervation at
the target may prove useful in preventing GID and in improving clinical
outcomes.\textsuperscript{46} In the end though, the utility of these protocol refinements
can be determined only through long-term follow-up studies ideally
incorporating a multi-modal imaging strategy.

**Conclusion**

Neuroimaging provides a unique means of assessing graft survival and
functional recovery in clinical trials of cell transplantation for PD.
Embryonic nigral DA tissue can be safely transplanted into the
putamen of patients with advanced PD. Blinded controlled studies in
conjunction with functional brain imaging have demonstrated long-
term benefit from these procedures. Although the magnitude of the
clinical response to transplantation is similar to that achieved by other
neurosurgical interventions, a continuous improvement of motor symp-
toms for over a decade has been reported in patients following fetal
cell transplantation with several open-label studies. Nonetheless,
further technical refinement of cell replacement strategies as we as
clinical investigation is needed to avoid the potentially disabling side
effect of GID in some cases. Given the positive long-term clinical
effects of fetal cell transplantation in PD, the ongoing European clinical
trial of this therapy and continual innovations and improvements in the
development of stem cell-generated dopamine neurons, DA cell trans-
plantation may gain currency as a viable treatment for PD patients.

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