Neuronal transplantation and its implication in Parkinson’s disease

Cell replacement technique where fresh neural tissues or genetically programmed cells are transplanted into the brain represents one of the most advanced procedures to repair damage and restore function in the lesioned or diseased brain. It however, does not represent a cure for the disorder but prevents or limits further degeneration thereby ameliorating the dysfunction.

Essential elements of neuronal transplantation

A) **Donor age**: Transplanted tissues of the CNS survive best when the cells are embryonic or in an active state of embryogenesis. Conversely, survival of the graft is found to be compromised when tissues were obtained after differentiation of the neurons. Thus, most neuronal transplantation studies use donor tissue prepared from animals during active neurogenesis in the region of interest.

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(Periods of neuronal histogenesis and the time windows that are typically used for suspension grafts)

B) **Host age**: The age of the host does affect to some extent the survival of the graft but has a major adverse effect on the graft fiber outgrowth particularly when the host is quite old. Whether these changes are due to the alterations in the levels of positive or negative growth factors or some other reasons remain to be identified.

C) **Vascularization**: The development of adequate blood supply to the graft is considered to be one of the most important factors determining the survival of the graft. Usually blood vessels invade the transplants as early as 24 hr, show high proliferation around 72 hr and then establish complete networks within 1 week postimplantation.

D) **Immunologic factors**: The brain is considered to be an “immunologically privileged” site. This does not refer to a lack of immune response but rather to the delayed responsiveness of the immune system to the implantation. Also, it is to be noted that immune response depends quite a lot on the origin of the donor tissue.

E) **Target access**: Appropriate target area greatly increases survival of the graft whereas placement of the graft in an inappropriate area drastically reduces the survival and growth of the graft. Interestingly, denervations have been found to increase graft survival and outgrowth.
**Cell types and methods of neuronal transplantation**

One important feature in transplantation procedure is that grafted tissue should be placed in target site/terminal area and not in the area of cellular origin. Grafts implanted at the cellular site survive but don’t extend processes over long distances to reinnervate the denervated target areas. At present following three methods of transplantation are being used:

**A) Transplantation of embryonic tissues:** In this case two different procedures are usually used for transplantation of the fetal neurons into the CNS; i) “solid-pieces” of tissues into a pre-formed cavity in the host brain ii) “cell suspension” in microlitre aliquots into multiple target sites in the host brain. Both procedures provide more or less similar results but are associated with following limitations; i) ethical issues of using fetal tissue and ii) availability of fresh tissue.

![Fig. 1: Schematic illustration of the two procedures used for septal grafting. A; tissues rich in ACh (tinted area) are dissected from the forebrain area of the embryonic brain (E15-E16). B; A coronal section through the forebrain shows the area containing the developing ACh neurons of the septal and diagonal bands (C). D; The transplants may be made by implanting solid pieces of tissue to the septal pole of the differented hippocampus. D; Alternatively, the graft tissue from many embryos can be pooled and then digested to form a cell suspension. The tissue is then injected in microlitre aliquots into multiple target sites (such as hippocampus, E; or neocortex, F) in the adult host brain.](image)

**B) Transplantation of genetically modified cells:** Two major groups of cells have been successfully used for gene transfer application to the CNS; i) immortalized cells and ii) primary cells. Transfer of essential gene or DNA into these cells is usually done using a variety of methods. The most commonly used method of gene transfer is retroviral infection - a technique that yields a relatively high efficiency of transgene incorporation into the host genome. Two classes of genes are of particular interest for CNS grafting; i) genes for growth factors - that are essential for regeneration and/or preventing degeneration of neurons, ii) genes that encode affected neurotransmitter/modulator specific enzymes.

The disadvantage of genetically modified cells or cell lines is that they secrete their product constitutively in an unregulated manner. The grafted neurons, on the other hand, establish synaptic contacts with the host neurons and are equipped with advanced functional regulatory systems.
C) Transplantation of polymer-encapsulated cells: Transplantation of polymer-encapsulated cells is another means of delivering neurotransmitter/growth factors to discrete CNS sites. These capsules comprise a selective barrier that allows free transport of low molecular weight molecules (nutrients, oxygen, electrolytes) while restricting the passage of larger species (immunoglobulins, cells etc.). This procedure has some advantages as well as disadvantages over other methods.

Implication of neuronal transplantation
Cell transplantation to the brain is now a well established research tool for studying the development and plasticity of the CNS. However, the procedure has been used extensively, at the animal as well as human levels, in relation to Parkinson’s disease (PD).

A) Parkinson’s disease (PD): Parkinson’s disease is a neurodegenerative disorder characterized by the degeneration of dopaminergic neurons of the substantia nigra pars compacta projecting to the striatum. Behaviorally, PD is primarily characterized by akinesia, rigidity, tremor and postural abnormalities. Current pharmacological treatments of the disorder involve administration of L-DOPA, or the use of certain drugs (e.g., bromocriptine) in conjunction with L-DOPA to stimulate striatal dopamine levels. Although effective in the early stages, this treatment has limited long-term success and does not prevent ongoing neuronal degeneration. There are two different animal models of PD which have been used extensively for neural transplantation studies:

1) 6-hydroxydopamine (6-OHDA) lesioned rats: Unilateral injection of the neurotoxin 6-OHDA either into medial forebrain bundle or directly into the substantia nigra destroys dopaminergic neurons in one side. This induces stereotypic rotational behavior which can be demonstrated by pharmacologically challenging the animals with dopaminergic drugs. This rotational behavior is quantifiable and its diminution can serve as an index of the restitution of impaired function.
Transplantation of the fetal substantia nigral tissue into the striatum of the 6-OHDA lesioned rats has been shown to promote recovery of the drug-induced rotational behavior and some sensory motor deficits. These studies reported survival of fetal tissue for 2-7 months after grafting and found that reduced rotational behavior was associated with the graft growth in the host brain.

ii) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesioned animal model: Peripheral administration of MPTP, a byproduct of synthetic heroin, produces bilateral lesion of the nigrostriatal pathways which leads to neurochemical impairments and behavioral changes resembling those observed in PD. Transplantation of fetal nigral grafts into MPTP-lesioned African green monkeys produced a significant recovery from tremors, freezing and difficulty in initiating movements. This recovery was evident until 7-8 months of post transplantation period.

Besides nigral tissue, other cell types that have attracted interest as a candidate for transplantation in animal models of PD are a) the adrenal chromaffin cells, b) fibroblast genetically modified to express tyrosine hydroxylase, c) polymer-encapsulated PC12 or glial derived neurotrophic factor (GDNF) producing cells and d) embryonic stem cells. However, the extent of functional recovery observed following nigral tissue implants is typically greater than other cell types in 6-OHDA lesioned rats or in MPTP-treated primates.

Neuronal transplantation in PD patients
The first clinical trials with autologous transplants of adrenal medullary tissue in PD patients were undertaken in early 1980s in Sweden with only minimal benefits. However, Madrazo et al. (1987), subsequently placed the grafts in contact with the cerebrospinal fluid and reported significant improvement in motor and cognitive function that persisted for at least 1 year. This procedure has been replicated by several groups worldwide with variable success. However, autopsy results from some of the grafted patients revealed either few or no surviving cells within the graft. Additionally, the high levels of mortality and morbidity associated with this procedure were found to outweigh the modest and transient improvements observed in PD patients.
Subsequently, embryonic ventral mesencephalic tissues were used for transplantation into the brains of PD patients (over 300 patients). The published results showed that grafted dopaminergic neurons can survive and mature up to 8 years after surgery and can alleviate the debilitating tremors associated with the disease. However, these early studies were all open label with no placebo arm. The first double-blind placebo controlled trial of transplantation in PD patients which was published in 2001 showed some improvements in UPDRS (Unified Parkinson’s disease rating scale) score for one year in younger group of patients (<60 years). The older patients did not improve as a group, although individuals did. There were no changes in the placebo group.

More recently, another double-blind transplant trial in PD patients showed only limited benefit on clinical outcomes despite good graft survival as demonstrated by PET scanning and post-mortem examination. Dyskinesias were present in a significant number of transplanted patients which may possibly due to the poorly functioning transplants.

**Concluding remarks**
Grafting studies have clearly demonstrated that damaged CNS tissue will regrow under certain conditions and are able to restore a variety of cognitive, sensory, and motor abilities in animal models of neurological disease. It is believed that refinement of this technique will offer additional strategies for developing therapies for the treatment of neurological disease.

**References**