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Improving Neuro-Regeneration Efficiency in the Stem Cell Treatments of Parkinson's Disease: Fetal Tissue vs. Mesenchymal Stem Cell

Mohammad Reza Ghaemi

Panama College of Cell Science, Division of Blue Marble University,
C&H Tower, Great George St., Roseau, 00152, Commonwealth of Dominica

Abstract: ***This research reviews the treatment concept and procedures that are currently being practiced and studied on patients using stem cell grafts of fetal tissue as well as mesenchymal stem cells, alongside the nature of neurogenesis phenomenon in correlation with subventricular zone architecture in individuals with Parkinson's disease in order to evaluate which treatment procedure has a higher efficiency rate in terms of neuro-regeneration capacity. This research also suggests considering the condition of subventricular zone prior to stem cell transplant since the neurogenesis process in human brain is compromised due to already impaired structure of subventricular zone as a result of dopamine depletion and lack of growth factors' regulatory effect; a precondition that could improve the efficacy of stem cell therapies for treatment of Parkinson's disease.***

Keywords: Subventricular zone, Fetal tissue graft, Mesenchymal stem cells, Neural stem cells, Neurogenesis.

1. Introduction

Parkinson's disease is a progressive neurodegenerative disorder that worsens as the individual ages. People with Parkinson's disease suffer from a selective degeneration of neurons in the substantia nigra, which produce the neurotransmitter dopamine that is essential for normal movement. This condition often leaves patients with severe mobility problems. The standard treatment includes the drug levodopa (L-dopa) that compensates for dopamine depletion in the brain [1]. However, long-term use of such medication can result in serious side effects, e.g. dyskinesia that is abnormal involuntary movements. Stem cell treatment aims to replace the missing neurons with dopamine producing (dopaminergic) cells from fetal brains and very recently with mesenchymal stem cells derived from adult human tissues such as bone marrow and adipose tissue. In 1987 at Lund University in Sweden, the first human transplantation of fetal brain took place, where the technique was established in which fetal ventral mesencephalic allografts is used as a dopaminergic replacement therapy in Parkinson's disease [2]. Another option for stem cell therapy is the use of adult stem cells, mesenchymal stem cells (MSCs), which are rapidly gaining popularity; due to their unique characteristics of availability (in autologous setting), procedure safety (no tumor formation and no immunological reaction due to immunomodulatory potential of MSCs), being free of ethical dilemma (compared to fetal tissue), as well as their paracrine factor and dopamine neuron protection and repair capability [3].

Neurogenesis is a continual process occurring in two regions of the adult mammalian brain namely dentate gyrus (DG) of hippocampus that is essential for processes involved in learning and memory as well as subventricular zone (SVZ) of lateral ventricles that generates new neurons [4]. The neurogenesis phenomenon, in addition to Parkinson's disease itself resulting from depletion of dopamine-producing cells, is significantly reduced in the individual's aging brain; and of these two regions, the focal attention of this research is on subventricular zone that is correlated with Parkinson's disease condition where neural stem cells (NSCs) are generated and migrate through Rostral Migratory Stream (RMS) to Olfactory Bulb (OB) where they differentiate into dopamine-producing neurons [5], [6].

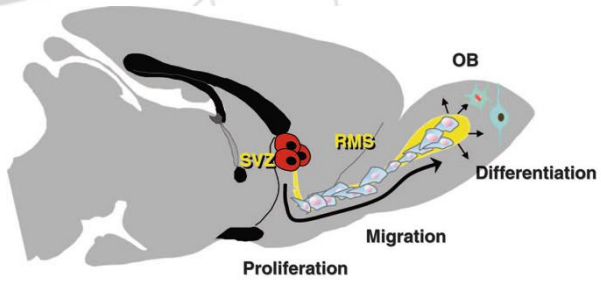


Figure 1: Subventricular Zone Neurogenesis Pathway

In the course of Parkinson's disease, the neurogenesis capacity of subventricular zone is impaired when dopaminergic midbrain neurons degenerate, leading to cerebral dopamine depletion. Dopamine is involved in the modulation of movements, mood and motivation. Dopamine also has been involved in regulating proliferation of neural stem cells (NSCs) in subventricular zone of the adult brain. Further, the growth factors are known to regulate and govern in vivo proliferation rate of NSCs, and of which epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2) are proven to synergically regulate the function of NSCs [7]. Nevertheless the growth factors in charge of regulating proportionally the dopamine level and SVZ neurogenesis phenomenon are produced by these NSCs and it is ostensible that low proliferation rate of NSCs would cause deficiency in growth factor production [8], which eventually would result in a missing bridge between the dopamine level and impaired SVZ infrastructure (Figure 2).

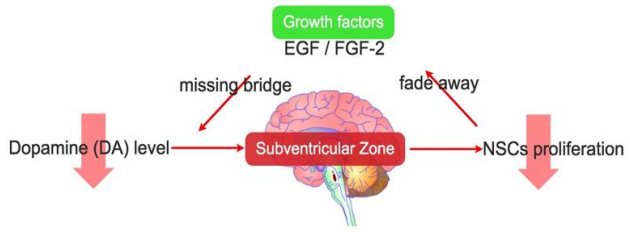


Figure 2: Disruption to regulatory effect of DA and Growth factors

The therapeutic objective is to improve neural activity in the degenerated area of brain through replacing dopamine-secreting cells like fetal mesencephalic tissue or very recently via mesenchymal stem cell grafts to compensate for selective loss of dopaminergic neurons in the substantia nigra. Effective transplant of fetal ventral mesencephalic cells, as the prominent treatment methodology proposed by leading scientific institutions in UK and US; could minimize both motor symptoms of Parkinson's disease as well as Levodopa-induced dyskinesia for a long period, and even decrease further need for dopamine replacement therapy. However, expected improvement would take long enough to be observed, in some cases around a year or even more after transplant; because newly replaced dopaminergic neurons require time (months to years) to mature and integrate into host brain, while in fact studies show that successful transplants can function and survive for many years [9]. Thus, one could speculate that the condition of subventricular zone - one of brain's two regions where neurogenesis occurs and is impaired in the course of Parkinson's - prior to fetal tissue transplant, might be the reason for such a long interval between the procedure performed and the improvements observed, which could be a deciding factor that will let researchers and physicians determine which stem cell therapy "Fetal tissue vs. MSCs" could be a better fit for treatment of Parkinson's disease in terms of neuro-regeneration efficacy with respect to two variables of time and clinical improvements.

2. Methodology

Database searches of national center for biotechnology information (NCBI) including PubMed category as well as Google Scholar were conducted in an iterative manner to retrieve articles related to Parkinson's disease and current stem cell treatment literatures with regards to neurogenesis and growth factor effects. The objective of this research review is to specify the characteristics of each stem cell treatment and to determine whether or not growth factors could have positive feedback on efficacy of these stem cell therapies for treatment of Parkinson's disease. Search terms included "stem cell treatment", "Parkinson's disease", "Subventricular Zone", "Epidermal Growth Factor", "Fibroblast Growth Factor-2", "Dopamine", "Mesenchymal stem cell", "Fetal tissue" and "Olfactory Bulb". A relatively small number of studies exist on human related topics, while studies principally are performed on non-human subjects such as mice and rodents as well as disease modelling on induced pluripotent stem cells (iPSCs); and therefore, a bottom-up search strategy was required. Furthermore, the reference list of each article was reviewed in detail for additional information.

3. Results and Discussion

3.1 SVZ Architecture and Parkinson's disease

Using electron microscopy, four different cell types have been identified that comprise subventricular zone based on ultra-structural characteristics observed. These four cells are astrocytes, transitory amplifying progenitors (also known as type C cells), neuroblasts and ependymal cells [10]. Among these cells a subpopulation of astrocytes has been identified that are slowly dividing and are known as neural stem cells [11]. The intermediary progeny of subventricular zone are type C cells that give rise to highly migratory neuroblasts organizing into chains that transit the subventricular zone and cross over rostral migratory stream (RMS) leading to olfactory bulb (OB) where neuroblasts commence their journey of differentiation into GABAergic and dopaminergic neurons at the final destination sites [5]. Figure "3" depicts the sequence of cell types involved in neuronal lineage and the specific markers that allow cell identification; as an example figure "4" illustrates the Immunocytochemical characterization of migratory neuroblasts (type A cells) in the subventricular zone using polysialic neural cell adhesion molecule (PSA–NCAM) and β tubulin III (TuJ1) staining. The chains of Type A cells are immunopositive for both PSA–NCAM and TuJ1 [10].

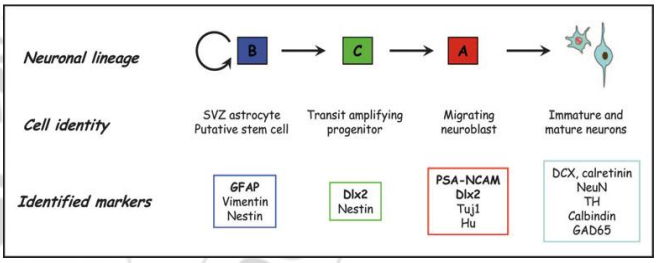


Figure 3: Sequence of cell types in neuronal lineage and respective markers

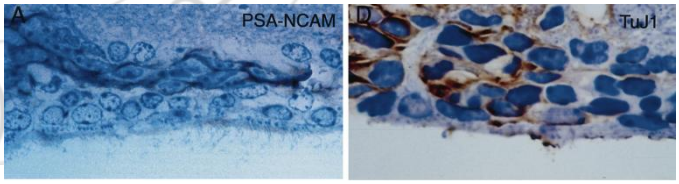


Figure 4: Immunocytochemical characteristic of type-A cells in SVZ

The fewer precursor cells (type C cells) are present in individuals with Parkinson's disease as a result of dopamine depletion. The C cells or NSC precursor cells express epidermal growth factor receptor (EGFR) that can be found in normal human (in this case an 84-year old male died with no neurological illness) between the ependymal cell layer and the striatum, which is illustrated in figure "5.a" using confocal microscopy where the close contact between dopaminergic fibers and $EGFR^+$ cells are preserved in the human brain [12]. The laser-scanning image (figure "5.b") demonstrates a dopamine transporter (DAT^+) nerve fiber contacting a cell of $EGFR^+$ that is surrounded by $GFAP^+$ astrocytic processes in the subventricular zone of a normal individual.

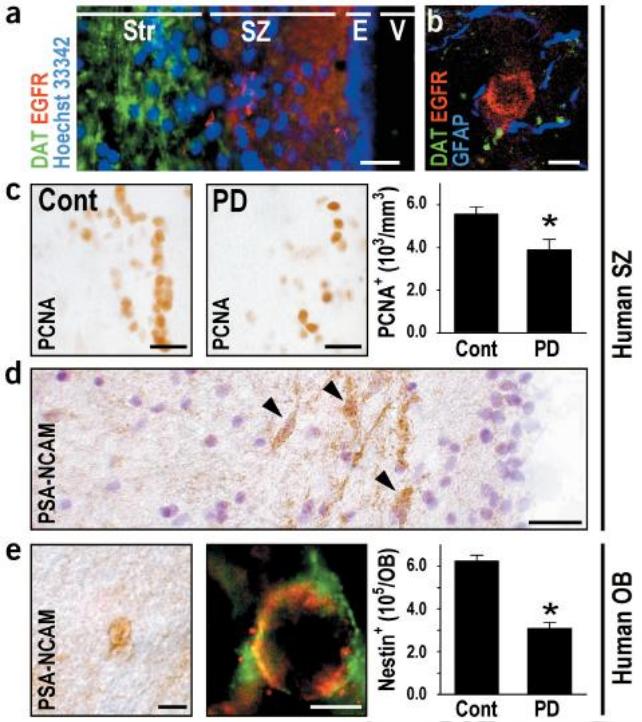


Figure 5: Precursor cells reduction in individuals with Parkinson's disease

The cell cycle marker, proliferating cell nuclear antigen (PCNA) was used to evaluate the influence of dopaminergic denervation on proliferation in human. The photomicrographs represented in figure "5.c" compares $PCNA^+$ cells in subventricular zone of a normal human that was used as a control subject with an individual with Parkinson's disease, in which $PCNA^+$ cells were decreased by 29.9% ($P<0.05$; t-test). Isolated PSA-$NCAM^+$ cells were also found in the human subventricular zone suggesting the presence of migrating cells sharing similarity with A-cells in animal model studies (figure "5.d"). It is also demonstrated that these PSA-$NCAM^+$ cells co-expressed nestin, which is an intermediate filament characteristic of immature neural precursors indicating that neural precursors exist in olfactory bulb of adult human (figure "5.e").

3.2 Fetal Tissue Transplant

In the late 1980s to the mid-1990s, a total of 18 patients received fetal cell transplantation in Lund, Sweden [2]. In this section, the rationale for use of fetal ventral mesencephalic allograft is discussed through long-term observation on two patients (number 7 and 15) from Lund experiments who received human fetal ventral mesencephalic tissue, rich in dopaminergic neuroblasts, as restorative treatment for their Parkinson's disease. The grafts (from aborted fetuses aged 6.5-9 weeks) were placed bilaterally using a magnetic resonance imaging-guided stereotactic technique for targeting putamen and caudate nucleus -adjacent to subventricular zone. Individuals were at ages of 49 (patient 7) and 54 (patient 15) years and after disease duration of 10 and 12 years, respectively [13].

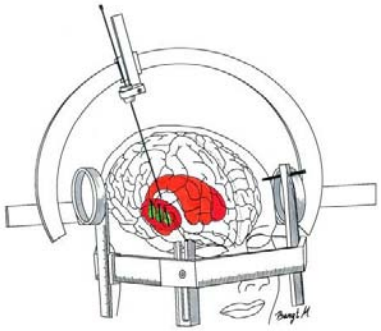
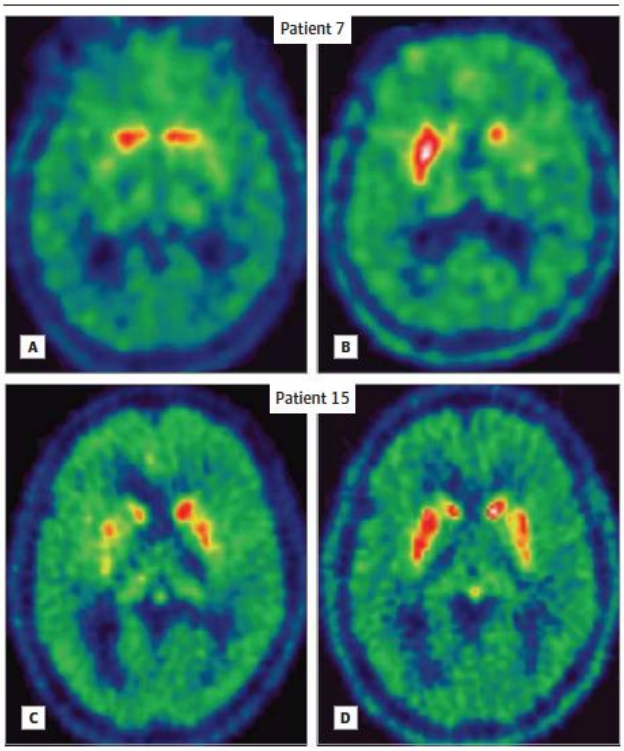


Figure 6: Example of a stereotactic transplantation of cells

Clinical assessments using the Unified Parkinson's Disease Rating Scale (UPDRS) were performed for motor function (part III) and Dyskinesia (part IV), 18 and 15 years of post-transplantation for patients 7 and 15, respectively. Individual number 7, following transplantation, experienced significant motor benefits, which gradually emerged over the course of four years. He was able to stop levodopa treatment 26 months after the first transplantation, by which time the "on-off" phenomena had virtually disappeared and his practically defined "off" motor UPDRS score had decreased by 38% compared with baseline score. By the fifth postoperative year, all dopaminergic agents had been withdrawn while the patient's motor status continued to improve. At his last assessment, 18 years post-grafting, the patient demonstrated sustained motor benefits scoring 22 on the UPDRS motor examination, reported no fluctuations, and remained free of any pharmacological dopamine replacement therapy.



Fetal mesencephalic grafts restored dopaminergic innervation in the striatum of 2 patients with Parkinson disease. The images portray striatal uptake before (A and C) and after (B and D) transplantation for patients 7 and 15.

Figure 7: Striatal ^{18}F-Dopa uptake increased after transplantation

Individual number 15, during the first 2 years after transplantation showed no improvement in UPDRS motor scores in the "practically defined off" state. However, "on" periods were prolonged and the patient's self-reported frequency and severity of motor fluctuations diminished allowing a 66% reduction in his daily levodopa equivalent dose. Improvements in motor function became more evident from the fourth year post-grafting, by the end of which he was able to stop all dopaminergic medication, given that "on" and "off" conditions were almost indistinguishable, and assessment 15 years post-transplantation demonstrated preserved motor benefits [13]. The sustained motor benefits agree well with the gradual complete normalization of ^{18}F-dopa uptake (as a measure of dopaminergic innervation) in the grafted putamen of both patients. Moreover, the course of Parkinson's disease is generally relentlessly progressive and the limited progression of disability in these two individuals after grafting is not usually achieved by other conventional therapeutic strategies. Figure "7" illustrates the 6-L-Fluorodopa F18 positron emission tomography images before and after the transplantation.

1) Ethical Issue

Such advancements have simultaneously raised profound ethical concerns and objections against the medical use of cadaveric fetal tissue, which is frequently derived from cases of elective abortion. The ethical debate in the United States, which involves anti-abortion movement, led to a moratorium on federal funding (1987-1992) of fetal tissue transplantation research. There are five issues related to fetal tissue transplantation. First, females may be advised or persuaded to undergo induced abortion on the grounds that it may help others by donating fetal tissue. Second, the widespread use of fetal tissue transplantations may result in an increase in the number of abortions. Third, the successful use of fetal tissue may make such procedures more socially acceptable. Fourth, the abortion procedure may be changed based on medical needs. Most notably, the question as to whether rightful informed consent for the use of fetal tissue can be obtained in cases of induced abortion is the most controversial issue. Ideally, the decision to undergo induced abortion should be completely separate from the consent to fetal tissue donation [14].

2) Side Effect

Graft-induced dyskinesias (GIDs), as the side effect of procedure, occurred in both patients but did not have a significant functional impact and their occurrence have been outweighed by the beneficial effects on motor functions, which was gained over the years. Nevertheless, GIDs represent a serious adverse event that can be debilitating but seem to be helped by deep brain stimulation of the internal segment of the globus pallidus [2]. Neither patient has been sufficiently troubled by their GIDs to consider pallidal deep brain stimulation as a treatment. Undoubtedly, understanding and finding ways to avoid GIDs remains of major importance for the future of dopaminergic cell therapies.

3) Delayed Improvement and Nature of Fetal Grafting

The gradual emergence of clinical improvements in both patients, although anticipated given the nature of the intervention (graft maturation and integration period); can be related to irregular dopamine level and especially disrupted growth factors' governing effects. Experimental studies in a 6-hydroxydopamie-induced rodents demonstrate that combined intraventricular infusion of EGF and FGF-2 induce massive increase in cell proliferation and in number of neuroblasts in subventricular zone. In addition, further studies indicate that adult neural precursor cells may be recruited from neurogenic regions to migrate toward lesion sites. For example, newly generated neural stem cells migrate from the subventricular zone into the ischemic striatum and express markers of developing and mature striatal spiny neurons. Interestingly, after global ischemia, recruitment of new hippocampal pyramidal neurons is facilitated by intraventricular infusion of epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2) [15]. Therefore, one can correlate the delayed improvements of fetal tissue transplant with the diminished level of EGF and FGF-2 that is incurred over the course of Parkinson's disease; when dopamine levels drop followed consequentially by low proliferation rate of neural stem cells (in SVZ) that release these two growth factors in response to dopamine stimulation via the protein kinase C signaling pathway [8]. The lack of growth factors' regulatory effect among series of impairments could explain the delay in patient's recovery result, where grafted dopaminergic neuroblasts need extended period of time to become mature within microenvironment of subventricular zone (SVZ) that is already experiencing deficiency of Epidermal growth factor and FGF-2, the two potent factors that increase the proliferation and mediate long-term survival of SVZ-derived progenitor cells; in addition to their capability to enhance dopaminergic neurogenesis in the olfactory bulb to become differentiated and redirected to the region of dopaminergic deficit [15].

3.3 Mesenchymal Stem Cells

The use of mesenchymal stem cells for tissue healing and regenerative medicine has been extended in the last decade due to their unique capabilities of multipotency (the ability to differentiate into tissue specific cells), immunomodulatory potential and their paracrine factors. Mesenchymal stem cells (MSCs) were originally identified in the bone marrow (BM); representing <0.01% of all nucleated BM cells, and now it has been found that they can be isolated from other tissues including adipose tissue, cord blood, peripheral blood, fetal liver, skeletal muscle, placenta, amniotic fluid and human brain. MSCs are characterized by expression of the surface markers CD73, CD90 and CD105, and they are negative for CD11b, CD14, CD19, CD79a, CD34, CD45 and HLA-DR. They are multipotent stem cells, which are capable of differentiating under appropriate condition into chondrocytes, skeletal myocytes, and neurons. Further, the MSCs can be harvested easily from adipose tissue or bone marrow of the patient's own body, expanded on the large scale for treatment of Parkinson's disease as these adult stem cells can transdifferentiate into neural cells following transplantation [3].

1) Immunomodulatory Effect

Human mesenchymal stem cells (hMSCs) display immune regulatory properties, which is an essential role in the therapeutic application of these cells. Both in vitro and in vivo studies using hMSCs demonstrated that these cells suppress the proliferation of T cells. Further, it has been revealed that hMSCs inhibit the differentiation and maturation of dendritic cells (DCs) and decrease the production of inflammatory cytokines by various immune cell populations. DCs are the most potent antigen-presenting cells, which specialize in antigen uptake, transport, and presentation and have the unique capacity to stimulate naïve and memory T cells. For instance, hMSCs facilitate engraftment of hematopoietic stem cells and prolong skin allograft survival. In addition, it has been demonstrated that the use of hMSCs reversed severe acute graft-versus-host-disease (GvHD). When undifferentiated hMSCs were transplanted into recipients in preclinical and clinical trials, these cells produced various cytokines and growth factors and had an ability to modify the response of immune cells and escape immune recognition (low immunogenicity). This indicates that, in addition to the transplantation of autologous cells to patients to minimize the risk of immune rejection, allogeneic hMSCs could also be used safely [16], [3].

2) Paracrine Action

hMSCs inherently synthesize and secrete a broad range of bioactive agents such as cytokines and growth factors. This intrinsic secretory activity of hMSCs significantly contributes to tissue repair or regeneration, presumably by establishing a regenerative microenvironment at sites of tissue injury or damage, while originally; the therapeutic effects observed with the use of hMSCs were thought to be due to their transdifferentiation (e.g. differentiation into non-mesodermal cell types) potential. It has been reported that following grafting into Parkinsonian rat brains, human bone-marrow derived MSCs (hBM-MSCs) differentiate into glial cells and secret an array of growth factors and cytokines including nerve growth factor, insulin-like growth factor-1 (IGF-1), FGF-2, and EGF that elicit neurotropic and neuro-protective effects on dopamine (DA) neurons; further, it is suggested that MSCs can exert their neuro-protective effect without direct cell contact, and therefore, engraftment of MSC is not necessary to protect neurons from inflammation, and therapeutic effects can even be obtained when MSC are administered via routes of delivery that place the cells at a distance from the target structure [17], [16]. Compared to paracrine effects, capacity of MSCs to transdifferentiate into neurons and fuse with host neurons is in less extent involved in their beneficial effects for Parkinson's disease.

3) MSC Transplantation

A surgical procedure, in which under short propofol anaesthesia bilateral frontal burr holes are drilled to access the subventricular zone via a standard brain cannula with Cosman-Roberts-Wells (CRW) stereotactic frame or Stealth (Medtronic) navigation assistance. MSCs is transplanted into the patient's brain at a dose of 2 million cells/kg body weight and gel-foam is placed over the dural defect prior to closing the wound. The patient is observed after surgery for 24 hours in intensive care unit and discharged home within 4-5 days. The described procedure is based on the study conducted by N. K. Venkataramana in both autologous and allogenic settings using bone marrow-derived MSCs (BM-MSCs) respectively performed in 2010 and 2012, which in the latter study; 12 individuals with Parkinson's disease, diagnosed between 5 to 15 years; were recruited and received allogenic BM-MSCs bilaterally into subventricular zone. Using unified Parkinson's disease rating scale (UPDRS) after treatment as the evaluating system; a mean improvement of 17.92% was reported during "on" and 31.21% during "off" period and none of the patients increased medication during the twelve-month follow-up period [18].

4) Systemic Administration

This procedure of systemic infusion of MSCs (e.g. intravenous administration) is based upon the hypothesis that MSCs are able to actively migrate across blood-brain barrier (BBB) that separates blood and brain in order that transplanted MSCs can home to and engraft at injured site in the brain to apply their therapeutic effects. The BBB, which is formed by cellular interactions between brain microvascular endothelial cells, astrocytes, pericytes and neuronsplays, plays a critical role in brain homeostasis by restricting the passage of molecules and leukocytes into and out of the brain; however, during brain inflammation and injury, the BBB becomes compromised and cellular trafficking through the BBB is significantly upregulated and leukocytes migrate across the endothelial barrier through both paracellular and transcellular pathways by a multistep adhesion/migration cascade, along with MSCs that can utilize a leukocyte-like, multistep homing cascade to engage with endothelia cells in order to transmigrate the barrier [19].

Figure 8 - MSCs therapeutic effects that can protect and stimulate regeneration in host-damaged DA neurons

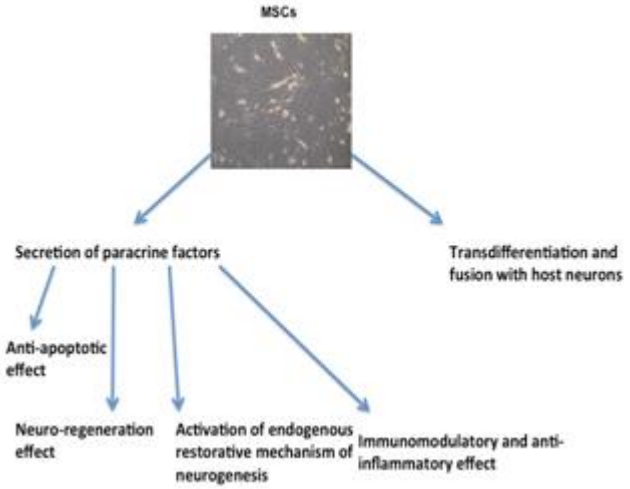


Figure 8: MSCs therapeutic effects that can protect and stimulate regeneration in host-damaged DA neurons

Figure "8" represents possible mechanisms that are involved in MSCs therapeutic effects for Parkinson's disease, in which they can protect and stimulate regeneration in host-damaged dopamine neurons, mainly through secretion of growth factors, cytokine, extracellular matrix proteins (ECM), and neuro-regulatory molecules that have the

capacity to decrease loss of dopamine neurons (anti-apoptotic effect) and create a favorable environment for neuronal regeneration. Through release of paracrine factors, MSCs are also able to affect the host tissue and facilitate restorative processes such as neurogenesis. Immunomodulatory and anti-inflammatory properties of MSCs are also implicated in their ability to protect and repair dopamine neurons.

5) Patient Testimonial Videos

Being relatively recent methodology, the therapeutic application of MSCs via systemic infusion requires to be evaluated first through comments of patients and caregivers. Therefore, the clinical outcomes are on patient narrative basis, where individuals describe their own experience, through which physicians and health professionals can adapt their models and delivery systems accordingly. The following videos are patient results, which have been published by treatment centers using MSCs derived from BM and/or adipose tissue and administrated via systemic infusion. In fact that was such cumulative evidence that led to the clinical trial using adipose-derived MSCs for treatment of Parkinson's disease, which is being currently conducted by U.S. National Institute of Health in collaboration with Stem Genex Medical Group. Please see anecdotal evidence section on table "1" for further details.Furthermore, the following table summarizes some of the institutions specialized in MSC treatment procedure. The selection of these institutions has been made in accordance with the essential questions asked on table "1" caption, the guidelines and procedural protocol performed in current research papers as well as the information presented on each institution's website. Because no treatment as such has been yet approved by U.S. Food and Drug Administration (FDA) due to the fact that this governing body considers stem cells, including individual's own cells, as medical products (drug) requiring specific regulations [20]; a long bureaucratic process over an un-patentable entity aside from scientific facts and findings.

Table 1: Institutional appraisal of adult stem cell treatment centers. The criteria for selection of treatment centers have been based on essential questions that should be accounted for before approaching such therapies; (1) What is being transplanted (type and source of stem cell)? (2) What is the proposed mechanism of action? (3) What are the clinical safety measurements with regards to ex vivo stem cell culturing and proliferation procedure (especially the type of medium used)? Based on available records and supporting data, and (4) What is being claimed with regards to potential therapeutic value of stem cell treatment? In accordance with anecdotal evidence described by patients or their caregivers,

Institution	*Location*	*Type of stem cell injected*	*Dose administrated*	*Autologous/ Allogenic*	*Anecdotal evidence by patient/caregivers*	*Remarks*
Stem Genex Medical Group	USA, California	Adipose-derived MSCs (AD-MSCs)	60 million	Autologous	- Following stem cell injection rigidity had reduced and movement had improved. - Improvements observed within almost immediately to a few months later (up to 6 months). Video link: https://stemgenex.com/studies/parkinsons-stem-cell-studies/parkinsons-stem-cell-treatment-warren-h/	- Patient's own cell is used (No host immune rejection) via mini-liposuction procedure - MSCs administrated directly into vein or via Intra Nasal to encourage more stem cells to travel past the Blood Brain Barrier (BBB). - 1-day minimally invasive procedure of a 3day program. - Repeated therapy recommended for cumulative effect from multiple stem cells - Recently conducted a clinical trial via U.S. National Institute of Health with anticipated date of completion on June 2017. https://clinicaltrials.gov/ct2/show/NCT02184546?term=stemgenex&rank=2

Swiss Medica Clinic	Switzerland	AD-MSCs / Bone Marrow derived MSCs (BM-MSCs) Cultured in Autologous serum (PRP)	200-300 million	Autologous	- After therapy the patient and relatives noticed a decrease in involuntary movements. Stiffness and tremor has reduced. - Patient is more independent in his daily activities Video link 1: http://www.startstemcells.com/parkinsons-treatment-stem-cells.html Follow-up video link 2: http://www.startstemcells.com/new-parkinsons-treatment.html	- 200ml adipose (fat) tissue is taken from patient's waist area (mini-liposuction) - Adipose tissue contains much larger volume of MSCs rather than BM. - MSCs are separated from fat in high-speed stem cell centrifuge machine, - Isolated MSCs are enriched in patient's own blood plasma, then photo activated in AdiLight machine that stimulates the release of growth factors from platelet-rich plasma (PRP) and activates stem cells, - Activated MSCs are administrated using standard IV drip. - 1-day minimally invasive procedure of 12day program.
STEMEDIX	USA, Florida	AD-MSCs Cultured in Autologous serum (PRP in the form of platelet lysate)	200-300 million	Autologous	- Rigidity and stiffness has reduced - Walking difficulty has also improved Video link: http://stemedix.com/parkinsons-disease/	- 200ml adipose tissue collected via mini-liposuction - Centrifuged to isolate dormant MSCs and then passed through anti-body filtration to ensure cell viability - Cultured and enriched in patient own PRP that includes growth factors - Pass through a laser process to bring MSCs from a resting to an activated state - Re-introduced back into patient's using several different methods, e.g. intravascularly (IV)
Repair Stem Cell Institute	USA (Texas. Florida) and Thailand	AD-MSCs BM-MSCs	60 million (x2)	Autologous	Reference: http://www.repairstemcells.org/Resources/Parkinson-s-Therapy-Comparison-Guide.aspx	- Primarily assembled as an advocating and advisory organization on adult stem cell treatment. - Double MSCs implementation via intravenous (IV) and intrathecal for better success rate with 2 days interval between procedures (each 60 million MSCs). - 6-month program is offered either in Florida (USA) or Thailand including nutritional consultation, acupuncture and physiotherapy.

6) Expansion of MSCs

MSCs have a rapid proliferation capability, achieving a thousand fold expansion of cell number in 2 to 3 week period. However, inappropriate expansion could reduce the quality of MSCs. It is known that extensive in vitro culture induces cellular senescence that is associated with growth arrest and apoptosis. In addition, particular therapeutic properties of MSCs could be lost during prolonged culture [21], [22]. Therefore, there are influential factors that must be considered in expansion of MSCs that will make a difference in clinical outcomes, which are detailed as per following table:

Table 2: Influential Elements in Expansion of MSCs

MSCs Expansion Elements	*Description*
Culture Flask	In order to obtain a sufficient number of MSCs for clinical application, a wide surface area is needed. Manufacturing companies such as Nunc (Denmark) and Corning (USA) offer large, multilayered culture systems that can fit to usual cell culture incubators, which reduce the number of culture flask used. The CellStacks (Corning) and CellFactory (Nunc) systems not only improve the microbiological safety and traceability but also reduce staff workload and cost.
Culture Medium	Consists of amino acids, glucose and ions including calcium, magnesium, potassium, sodium and phosphate. Dulbecco's Modified Eagle's Medium (DMEM) is preferably used with low glucose and Glutamax to substitute the L-glutamine that forms ammonia during its metabolic pathway inhibiting cell growth.
Growth Factor Supplements	Platelet lysate is used preferably from patient's own blood (buffy coats). Immediately after collection, platelet products are frozen at − 80∘ C and subsequently thawed to obtain the release of growth factors included in platelets with centrifugation to eliminate platelet bodies. The obtained growth factors include fibroblast growth factor-2 (FGF-2), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor (TGF)-β, and insulin-like growth factor (IGF-1).
Passaging and Storing	Trypsin-EDTA solution is used to dissociate adhered MSCs on plastic flasks through enzymatic digestion, for the purpose of passaging for expansion or collection for administration. Isolated and expanded MSCs are sometimes stored until the time of treatment. However, there are reports warning hazardous effects by cryopreservation due to important ingredient of dimethylsulfoxide (DMSO) in current freezing solution, which has a high membrane permeability and can be damaging to cells when used in high concentration. Also, if it remains in MSC suspension for administration, DMSO can cause adverse reactions in patients, including nausea, vomiting, tachycardia, bradycardia, and hypotension. Thus, physicians are advised to use fresh cells instead of cryopreserved cells. It is important to optimize the vehicle of MSCs for injection in order to increase the cell viability before and after injection. Therefore, in clinical application saline is used as an injection vehicle, while some researcher attempt to supplement human serum albumin to protect cells from environmental stress and prevent adherence to the walls of tubes and needles.

4. Conclusion

The current treatment for Parkinson's disease includes pharmacotherapies and deep brain stimulation techniques. However, these can only produce symptomatic relief and have their own limitations. Therefore, the need for alternative therapy is required. The table "3" provides readers with a comprehensive data that put these two described stem cell treatments in perspective to enable physicians and health professionals to select the appropriate stem cell treatment according to the patient's individual needs.

Table 3: Fetal Tissue Graft vs. MSCs application

Therapeutic Characteristics	*Fetal Tissue*	*Mesenchymal Stem cell*
Growth Factor Effect	Fetal tissue also release cytokines, however, the effect is implemented only after maturation and integration of graft into host when dopamine level upregulated to stimulate NSCs proliferation that eventually produce EGF and FGF-2 to maintains subventricular structure and consequently to improve neurogenesis process (Figure 2).	Yes, via capability of paracrine factors; MSCs are able to secrete various growth factors including EGF and FGF-2 that could promote their therapeutic effect.
Implementation Method	Surgical	Surgical (bilateral transplant), and/or Non-invasive (e.g. IV drip)
Immunosuppressive Drug	No, because cellular antigens are not expressed during the fist trimester [23]; however, in a/m clinical trial as precaution cyclosporin was administrated.	No. While the focal attention of this research is mainly on Autologous procedure setting, due to immunomodulatory property of MSCs; even in Allogenic setting the immunosuppression is not required and no tumor is formed.
Result Pending Period	Successful outcome takes about a year to be observed onwards, and the delay is linked with this research hypothesis, which concerns lack of EGF/FGF regulatory effect caused by SVZ structural impairment due to nature of PD condition.	Successful outcome is observed within weeks to months, and even earlier in some cases according to available anecdotal evidence.
Treatment Efficacy compare to Conventional Pharmacological Therapy	Dopaminergic cell replacement will only ever work as well as the best dopaminergic agents, such as levodopa. As such, they are not able to treat most of non-motor features of Parkinson's disease, many of which are non-dopaminergic in origin. Therefore, cell treatments aimed at dopamine cell replacement are not curative; in the same way that levodopa is not curative. But, if used early, they could substantially reduce the amount of medication needed by the patient and, therefore, strikingly alter the natural history of treated Parkinson's disease.	Caregivers have noticed an overall improvement in the activity level of patients, which includes reduction in tremors both at rest and in motion, better clarity in speech, reduction in rigidity, ability to walk for longer distances and perform personal tasks independently. This has a significant impact on the well being of the patients and further substantiates the fact that progression of the disease has been slowed down after MSC therapy.
Side-effects	In the initial open-label studies of fetal ventral	No dramatic side effect reported for systemic administration.

	mesencephalic transplants, various clinical benefits and no clinically significant side effects were reported. Subsequently, some patients developed graft-induced dyskinesias, the cause of which has been a matter of intense debate. These movements disorders were reported in only some individuals, all of whom had levodopa-induced dyskinesias before grafting.	For bilateral transplant, in the late stages of disease; the stem cell transplant had shown relatively lesser symptomatic relief and on the other hand needed an increase in medications.

The patient's age and the stage of the disease are two important factors to consider when utilizing MSCs. Venkatarmana et al. (2010) demonstrated that the autologous transplantation of BM-MSCs into subventricular zone had mixed results, in which some patients showed improvements but after initial improvement period, a deterioration of symptoms was followed. This was possibly due to the advanced stage of disease and the continued degeneration in the non-grafted region. In addition, there is a difference in the population doubling time (PDT), morphology, differential potential, and cell senescence during proliferation of MSCs. Although the study confirmed that the cells met the requirements of the international society for cellular therapy, the PDT, and cell surface marker expression and differential potential was observed to be lesser than healthy donor BM-MSCs. This may be attributed to the higher age of patient where cells are known to have shorter telomeres and lower proliferation potential; because these cells reached senescence ex vivo much earlier at passage no.3, and consequently made it challenging to be able to upscale the cells for transplantation. Thus, in order to prevent these shortcomings as much as possible, the systemic administration of MSCs can be implemented (instead of surgical transplant) where injected MSCs can pass through BBB and be distributed in more evenly and balance manner within the injured regions of the individual's brain, which is mediated by homing effect of MSCs; a therapeutic characteristic to migrate towards damaged tissues guided by chemokines released from the damaged brain and their receptors [3]. Further, to increase the quantity of viable adult stem cells; we can switch to adipose-derived MSCs (AD-MSCs) as an alternative that in fact contains approximately a 500-fold higher frequency of adult stem cells compared to bone marrow, and tissue collection is simple, and are more potent at suppressing the immune system suggesting their superiority in clinical therapies [24]. An additional, yet crucial step is to humanize the culture media for human MSCs by using autologous serum including patient's own platelet derivatives (e.g. platelet lysate). MSCs cultured with PRP in the form of platelet lysate will improve the cells proliferation rate since the platelets function as a natural reservoir for growth factors including EGF and FGF providing appropriate microenvironment for MSCs to expand while preserving their multipotency characteristic [25]. Furthermore, low-level laser irradiation has been shown to effectively increase the MSCs expansion, and the higher the proliferation rate, the greater the regeneration and healing capacity of the tissues; a process that is referred to photo-activation of dormant adult stem cells by specialists in this field. While the underlying molecular mechanisms for this process are not yet completely understood, It has been suggested that the energy of the laser is absorbed by intracellular chromophores and converted into metabolic energy, which is then used by the mitochondrial respiratory chain to produce ATP and consequently to increase DNA activity, the synthesis of RNA and proteins such as fibroblast growth factor and epidermal growth factor, when AD-MSCs are exposed to a laser at a wavelength of 635nm and dose of 5.0J/cm^2, which positively as well influence the cell proliferation and its viability [26].

4.1 Procedural Protocol and Technical Video

Based on the above explanation, the implementation of adipose-derived mesenchymal stem cells (AD-MSCs) is a more global approach to the problem, which would primarily assist in neuro-protection of affected region followed by neurogenesis to restore neural functions. The current protocol of AD-MSC therapy via systemic administration in autologous setting is mapped out in figure "9" and can be viewed using video link below:

- Technical video link: ttp://www.startstemcells.com/stem-cells-treatment-procedure.html

In this procedure 200ml of adipose tissue is harvested from patient's waist area through mini-liposuction procedure. Collected adipose tissue is incubated with collagenase type1 to enzymatically digest the fat and the sample is centrifuged to isolate the dormant MSCs from adipose tissue. Platelet-rich plasma (PRP) is obtained from the patient's own blood to provide MSCs with proper culture media for expansion. AdiLight instrument (AdiStem Ltd.) is used for 30-minute period to activate AD-MSCs through photo-modulation and to stimulate autologous PRP for growth factor release. The activated adult stem cells are administrated into the patient's body via several different methods depending on patient's medical history and the treatment plan chosen; which are intravascular (IV), nasal-cavity injection, intrathecal injection, nebulizer treatment, etc.

Figure 9: AD-MSCs Therapeutic Protocol in Autologous Setting

It is recommended that the individual should repeat the procedure at a certain interval (usually less than a year) to ensure a cumulative effect and a higher treatment efficacy. In the end, it is crucial to expose the degenerating brain to the exogenous stimulus of mesenchymal stem cells, while the in situ neuro-regenerative reservoir of stem cells is present particularly at subventricular zone in order to maintain

functional neurons through therapeutic application of MSCs that assist in neuroprotection and neurogenesis.

5. Future Scope

We can conclude from this review that both types of stem cell treatments of fetal tissue and MSCs mediate the endogenous production of growth factors (EGF and FGF-2). However, the effect of MSC application is facilitated by its unique therapeutic characteristic of paracrine action that has beneficial neuroprotective and neuro-restorative effects in patients with Parkinson's disease. This review advances the field of regenerative medicine through understanding how MSCs help in neuroprotection and neurogenesis. The next step is to experiment the direct injection of growth factors of EGF and FGF-2. However, the long-term consequence of over-stimulating the remaining NSCs in the elderly brain of individuals with Parkinson's disease is not known. Does this act to further exhaust the aged and impaired NSC pool? Ultimately, both the ability to stimulate and revitalize the subventricular zone architecture without compromising its future capacities has significant implications for regenerative medicine.

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Author Profile

Mohammad Reza Ghaemi began his career as a chemist and later on during postgraduate study; he pursued his passion in biochemistry at British Columbia Institute of Technology and University of Victoria (Canada) where he became interested in stem cell biology and its application in treatment of neurodegenerative diseases. He continued his education in stem cell biology at University of Ulster (UK) and participated at stem cell training workshops of University of Oxford, and pursued his research in adult stem cell application through Panama College of Cell Science where he received his PhD in stem cell biology.