ISOLATED CELLS AND POPULATIONS COMPRISING SAME FOR THE TREATMENT OF CNS DISEASES

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to cells and populations thereof which can be used for treating CNS diseases.

Parkinson's disease is an age-related disorder characterized by progressive loss of dopamine producing neurons in the substantia nigra of the midbrain, which in turn leads to progressive loss of motor functions manifested through symptoms such as tremor, rigidity and ataxia.

Current treatment strategies for PD focus on restoring the depletion of dopamine, generally through the administration of the dopamine precursor L-DOPA (L-3-4-dihydroxyphenylalanine). L-DOPA, (the blood-brain barrier (BBB) penetrating precursor of dopamine), successfully increases the synthesis and release of dopamine. However, as the disease progresses, less dopaminergic neurons are available to synthesize dopamine from the precursor and the effectiveness of the treatment decreases whilst L-DOPA-induced dyskinesia appears. Other treatments with dopamine agonists, monoamine oxidants inhibitor or COMT inhibitors also demonstrate partial improvement but they cannot prevent progression of the disease.

Cell transplantation has been suggested as an alternative treatment option for repairing and replacing missing dopaminergic neurons. For such cell replacement therapy to work, implanted cells must survive and integrate, both functionally and structurally, within the damaged tissue.

The use of stem cells as a cellular source in cell replacement therapy for Parkinson's disease has been recently suggested. Stem cells have the ability to exist in vivo in an undifferentiated state and to self-renew. They are not restricted to cell types specific to the tissue of origin, and so they are able to differentiate in response to local environmental cues from other tissues. This capability of self renewal and differentiation has great therapeutic potential in curing diseases.

In Parkinson's disease the stem cell replacement strategy is based on the idea that restoration of dopamine (DA) neurotransmission is effected by cell grafts that integrate over time into the remaining tissue and produce a long-lasting functional tissue. There are two methods of treating stem cells for grafting in PD. In the first method, prior to transplantation, cells are differentiated *in-vitro* to dopaminergic

neurons. This allows for standardization and quality-control of the relevant cells. The second method comprises transplantation of undifferentiated stem cells that are thought to differentiate *in- vivo* to dopaminergic neurons following implantation into the striatum or substantia nigra.

In theory, DA neurons for cell therapy in PD could be made from stem cells from four different sources: fetal dopaminergic neurons, neural stem cells, embryonic stem cells and bone marrow stem cells.

Bone marrow contains two major populations of stem cells: hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) occasionally referred to as bone marrow stromal cells.

Rat BMSC following differentiation were shown to express Tyrosine-hydroxylase (TH), choline acetyltransferase and beta-III tubulin [Woodbury, D., et al., J Neurosci Res. 69(6):908-17, 2002]. Clinical therapeutic potential of mouse BMSC in PD was demonstrated by injecting mouse BMSC intrastriatally to 1-metyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) mouse model of PD. The transplanted cells survived and expressed TH. Moreover improvement on the rotarod test at 35 days following transplantation was indicated [Li, Y., et al., Neurosci Lett. 316(2):67-70, 2001].

U.S. Patent Appl. 20050265983 to the present inventors teach human dopamine synthesizing MSCs which expressed neuronal markers and transcription factors that characterize midbrain DA neuron following induction of neuronal differentiation.

As an alternative to a cell replacement strategy, where the grafted cells have to survive and possess morphological electrophysiological and functional dopaminergic properties, cell therapy may be aimed at restoring or reestablishing the normal anatomy (connectivity) and physiology (appropriate synaptic contacts and functioning) of the striatum.

Neurotrophic factors (NTFs) are secreted proteins that regulate the survival, functional maintenance and phenotypic development of neuronal cells. Alterations in NTF levels are involved in triggering programmed cell-death in neurons and thus contribute to the pathogenesis of Parkinson's and other neurodegenerative diseases.

One of the most potent NTF for dopaminergic neurons is called glial cell line-derived neurotrophic factor (GDNF). It is known to promote the survival of the dopaminergic neurons in the substantia nigra, promote neurite outgrowth, increase

cell body size and also raise levels of TH. GDNF belongs to a family of proteins, related to the TGF-β-superfamily, currently consisting of four neurotrophic factors: GDNF, Neurturin (NTN), Persephin, and Artemin/Neublastin. These factors are known to serve as regulators of cell proliferation and differentiation.

An analysis of neural progenitor cells (ST14A) revealed that GDNF overproduction may be associated with up-regulation of genes involved in axonal sprouting, neurite outgrowth, spine formation, vesicle transport and synaptic plasticity [Pahnke J, et al, Exp Cell Res. 297(2):484-94, 2004]. It was also suggested that the neuroprotective activity of GDNF is via its activation of the antioxidant enzyme systems such as glutathione peroxidase, superoxide dismutase and catalase activities [Chao CC, Lee EH. Neuropharmacology, 38(6):913-6, 1999].

Various cells type produce GDNF including glia cells (oligodendrocytes and astrocyte), neuroblastoma and glioblastoma cell lines. It has recently been shown that rat BMSCs cultured in DMEM supplemented with 20% fetal bovine serum, at passage 6 express GDNF and NGF [Garcia R, et al., Biochem Biophys Res Commun. 316(3):753-4, 2004].

GDNF synthesis can be regulated by growth factors, hormones, cytokines and neurotransmitters. For example, tumor necrosis factor-α or interleukin-1 induces release of GDNF from glioblastoma cells. Forskolin or cAMP causes an increase in GDNF release by both the neuroblastoma and glioblastoma cell lines. These cells comprise neurotransmitter receptors, which allow the neurotransmitters to regulate growth factor production under conditions of stress.

Administration of GDNF directly into the brain has been shown to be effective in various animal models of PD. In addition, exposure of cells to GDNF prior to transplant has proven beneficial. For instance, grafting of 400,000 fetal dopaminergic neurons prior to transplantation significantly improved the rotational behavior of lesioned rats [Mehta V, et al., J Neurosurg. 1999 Apr; 90(4):804-6].

Various methods have been used to aid administration of GDNF into the brain including osmotic pumps, capsules and microspheres. Another approach for GDNF delivery is in vivo gene therapy. Bone marrow mesenchymal cells genetically engineered to express GDNF, transplanted into MPTP-lesioned mice, were able to protect nigral neurons as well as striatal fibers [Park, K., Neurosci. Res. 40: 315-323, 2001].

Several studies have shown that MSCs following exposure to different factors *in vitro,* change their phenotype and demonstrate neuronal and glial markers [Kopen, G.C., et al., Proc Natl Acad USA. 96(19):10711-6, 1999; Sanchez-Ramos, et al. Exp Neurol. 164(2):247-56. 2000; Woodbury, D., J Neurosci Res. 61(4):364-70,2000; Woodbury, D., et al., J Neurosci Res. 69(6):908-17, 2002; Black, I.B., Woodbury, D. Blood Cells Mol Dis. 27(3):632-6, 2001; Kohyama, J., et al. Differentiation. 68(4-5):235-44, 2001; Levy, Y.S. J Mol Neurosci. 21(2):121-32, 2003].

However, none of these studies have shown human MSCs capable of secreting significant levels of neurotrophic factors.

There is thus a widely recognized need for, and it would be highly advantageous to have, transplantable cells capable of synthesizing neurotrophic factors such as GDNF for the treatment of neurodegenerative disorders.

SUMMARY OF THE INVENTION

According to the present invention there is provided an isolated human cell comprising at least one astrocytic phenotype and at least one mesenchymal stem cell phenotype, wherein the mesenchymal stem cell phenotype is not an astrocytic phenotype.

According to another aspect of the present invention there is provided an isolated human cell comprising at least one mesenchymal stem cell phenotype and at least one astrocytic structural phenotype, wherein the mesenchymal stem cell phenotype is not an astrocytic structural phenotype.

According to yet another aspect of the present invention there is provided an isolated human cell comprising at least one mesenchymal stem cell phenotype and at least one astrocytic functional phenotype, wherein the mesenchymal stem cell phenotype is not an astrocytic functional phenotype.

According to still another aspect of the present invention there is provided an isolated human cell comprising at least one mesenchymal stem cell phenotype and expressing at least one neurotrophic factor, wherein the expression is at least 2 times greater than a basal expression of the neurotrophic factor in a mesenchymal stem cell.

According to an additional aspect of the present invention there is provided an isolated cell population comprising human cells wherein:

(i) at least N % of the human cells comprise at least one astrocytic phenotype;

(ii) at least M % of the human cells comprise at least one mesenchymal stem cell phenotype, the mesenchymal stem cell phenotype is not an astrocytic phenotype; and(iii) at least one of the human cells comprises both the at least one astrocytic phenotype and the at least one mesenchymal stem cell phenotype;

where M and N are each independently selected between 1 and 99.

According to yet an additional aspect of the present invention there is provided an isolated cell population comprising human cells wherein:

(i) at least N % of the human cells express at least one neurotrophic factor, wherein the expression is at least 2 times greater than a basal expression of the neurotrophic factor in a mesenchymal stem cell;

(ii) at least M % of the human cells comprise at least one mesenchymal stem cell phenotype; and

(iii) at least one of the human cells expresses the at least one neurotrophic factor and the at least one mesenchymal stem cell phenotype;

where M and N are each independently selected between 1 and 99.

According to still an additional aspect of the present invention there is provided an isolated cell population comprising human cells wherein:

(i) at least N % of the human cells comprise at least one astrocytic structural phenotype;

(ii) at least M % of the human cells comprise at least one mesenchymal stem cell phenotype, the mesenchymal stem cell phenotype is not an astrocytic structural phenotype; and

(iii) at least one of the human cells comprise both the at least one astrocytic structural phenotype and the at least one mesenchymal stem cell phenotype;

where M and N are each independently selected between 1 and 99.

According to a further aspect of the present invention there is provided an isolated cell population comprising human cells wherein:

(i) at least N % of the human cells comprise at least one astrocytic functional phenotype;

(ii) at least M % of the human cells comprise at least one mesenchymal stem cell phenotype, the mesenchymal stem cell phenotype is not an astrocytic functional phenotype; and

(iii) at least one of the human cells comporise both the at least one astrocytic functional phenotype and the at least one mesenchymal stem cell phenotype;

where M and N are each independently selected between 1 and 99.

According to yet a further aspect of the present invention there is provided a method of generating astrocyte-like cells, comprising incubating mesenchymal stem cells in a differentiating medium comprising platelet derived growth factor (PDGF) and human neuregulin 1-β1, thereby generating astrocyte-like cells.

According to still a further aspect of the present invention there is provided a method of generating astrocyte-like cells, comprising incubating mesenchymal stem cells in a medium comprising at least one differentiating agent, the at least one differentiating agent being selected from the group consisting of platelet derived growth factor (PDGF), human neuregulin 1-β1, FGF2, EGF, N2, IBMX and cAMP, thereby generating astrocyte-like cells.

According to still a further aspect of the present invention there is provided a method of treating a CNS disease or disorder comprising administering to an individual in need thereof a therapeutically effective amount of astrocyte-like cells, thereby treating the CNS disease or disorder.

According to still a further aspect of the present invention there is provided a use of astrocyte-like cells for the treatment of a CNS disease or disorder.

According to still a further aspect of the present invention there is provided a pharmaceutical composition comprising as an active agent any of the cells or cell populations of any of claims 1, 2, 3, 4, 5, 6, 7 or 8 and a pharmaceutically acceptable carrier.

According to further features in preferred embodiments of the invention described below, the cells are non-genetically manipulated.

According to still further features in the described preferred embodiments, the at least one astrocytic phenotype is a structural phenotype.

According to still further features in the described preferred embodiments, the at least one astrocytic phenotype is a functional phenotype.

According to still further features in the described preferred embodiments, the cells further comprise an astrocytic functional phenotype.

According to still further features in the described preferred embodiments, the astrocytic functional phenotype is not the mesenchymal stem cell phenotype.

According to still further features in the described preferred embodiments, the cells further comprise an astrocytic structural phenotype.

According to still further features in the described preferred embodiments, the cells further comprise an astrocytic structural phenotype.

According to still further features in the described preferred embodiments, the astrocytic structural phenotype is not the mesenchymal stem cell phenotype.

According to still further features in the described preferred embodiments, the astrocytic structural phenotype is a cell size, a cell shape, an organelle size and an organelle number.

According to still further features in the described preferred embodiments, the astrocytic structural phenotype is expression of at least one astrocytic marker.

According to still further features in the described preferred embodiments, the astrocytic marker is a surface marker.

According to still further features in the described preferred embodiments, the astrocytic marker is an internal marker.

According to still further features in the described preferred embodiments, the astrocytic functional phenotype is expression of at least one neurotrophic factor at a level at least 2 times greater than a basal production of the neurotrophic factor in a mesenchymal stem cell.

According to still further features in the described preferred embodiments, the at least one neurotrophic factor is selected from the group consisting of glial derived neurotrophic factor (GDNF), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4/5, Neurturin (NTN), Persephin, brain derived neurotrophic factor (BDNF), artemin (ART), ciliary neurotrophic factor (CNTF), insulin growth factor-I (IGF-1) and Neublastin.

According to still further features in the described preferred embodiments, the at least one neuroptrophic factor is GDNF.

According to still further features in the described preferred embodiments, the astrocyte marker is selected from the group consisting of S100 beta, glial fibrillary acidic protein (GFAP), glutamine sythetase, GLT-1 and GLAST.

According to still further features in the described preferred embodiments, the secretion of the GDNF is regulated by IL-1 beta and/or cabergoline.

According to still further features in the described preferred embodiments, a duration of the incubating is about 48 hours.

According to still further features in the described preferred embodiments, a concentration of the PDGF is about 5 ng/ml.

According to still further features in the described preferred embodiments, a concentration of the human neuregulin 1-β1 is about 50 ng/ml.

According to still further features in the described preferred embodiments, the differentiating medium further comprises L-glutamine, dibutyryl cyclic AMP and isobutylmethylxanthine IBMX.

According to still further features in the described preferred embodiments, the method further comprises culturing the cells in an additional medium prior to the incubating thereby predisposing the cells to differentiate into astrocyte-like cells.

According to still further features in the described preferred embodiments, the additional medium comprises human epidermal growth factor (hEGF) and human basic fibroblast growth factor (hbFGF).

According to still further features in the described preferred embodiments, a concentration of hEGF is about 20 ng/ml.

According to still further features in the described preferred embodiments, a concentration of hbFGF is about 20 ng/ml.

According to still further features in the described preferred embodiments, the additional medium further comprises L-glutamine, insulin, progesterone, putrescin, selenium and transferrin.

According to still further features in the described preferred embodiments, a duration of the culturing is about 48 hours.

According to still further features in the described preferred embodiments, the mesenchymal stem cells are obtained by: (a) culturing a population of cells comprising the mesenchymal stem cells in a proliferating medium capable of maintaining and/or expanding the mesenchymal stem cells; and (b) selecting mesenchymal stem cells from the cells resulting from step (a).

According to still further features in the described preferred embodiments, the step (b) is affected by harvesting surface adhering cells.

According to still further features in the described preferred embodiments, the method further comprises administering to the individual, stem cells capable of endogenously synthesizing at least one neurotransmitter.

According to still further features in the described preferred embodiments, the CNS disease or disorder is a neurodegenerative disease or disorder.

According to still further features in the described preferred embodiments, the CNS disease or disorder is selected from the group consisting of a motion disorder, a dissociative disorder, a mood disorder, an affective disorder, an addictive disorder and a convulsive disorder.

According to still further features in the described preferred embodiments, the neurodegenerative disorder is selected from the group consisting of Parkinson's, multiple sclerosis, epilepsy, amyatrophic lateral sclerosis, stroke, autoimmune encephalomyelitis, diabetic neuropathy, glaucatomus neuropathy, Alzheimer's disease and Huntingdon's disease.

According to still further features in the described preferred embodiments, the cells are autologous cells.

According to still further features in the described preferred embodiments, the cells are non-autologous cells.

The present invention successfully addresses the shortcomings of the presently known configurations by providing cells and populations thereof capable of secreting neurotrophic factors.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it

is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIGs. 1A-B are light microscopy images of 5 day-differentiated and non-differentiated human MSCs. The differentiated human MSCs demonstrate astrocyte-like morphology such as the typicalastro-like structure. (light microscopy).

FIGs. 2A-D are scanning electro-microscopy images of 5 day-differentiated (Figures 2B-D) and non-differentiated (Figure 2A) human MSCs. Differentiated human MSCs demonstrate astrocyte-like morphology.

FIGs. 3A-B are photomicrographs illustrating the expression of astrocyte markers in the differentiated human MSC Immunostaining was preformed on differentiated human hBMSc with anti- S100β (Figure 3A), and anti-glutamine synthetase (Figure 3B). Cell nuclei were stained using DAPI (blue).

FIGs. 4A-B are photomicrographs are bar graphs illustrating the expression of the astrocyte marker glial fibrillary acidic protein (GFAP) in the differentiated human MSCs. Immunostaining was performed on differentiated human hBMSc with anti-GFAP and cell nuclei were stained using DAPI (blue) (Figure 4A). Figure 4B is a bar graph illustrating the level of GFAP in cell extracts of the differentiated human MSCs as analyzed by real time PCR using primers SEQ ID NOs: 11 and 12.

FIG. 5 is a bar graph illustrating the amounts of neurotrophic factors transcripts in differentiated human MSCs compared to non-differentiated human MSCs. Cell extracts were subject to real time PCR assay for GDNF (SEQ ID NOs: 3 and 4), NGF (SEQ ID NOs: 9 and 10) and BDNF (SEQ ID NOs: 5 and 6) transcripts.

FIGs. 6A-B are photomicrographs and bar graphs illustrating the expression of GDNF on the differentiated human MSCs. Immunostaining was performed on differentiated human hBMSc with anti-GDNF. Cell nuclei were stained using DAPI (blue) (Figure 6A). Figure 6B illustrates that the number of the cells that positively

stained with anti-GDNF was about 30 % (*P < 0.05, n = 3, average cells counted per n = 415.5 ± 37.5). The number of GDNF cells was evaluated by examination of 15 fields from three independent cultures. The total number of cells in these samples was determined by counting DAPI stained cell nuclei and results are expressed as mean ± SEM percentages of positive cells.

FIG. 7 is a bar graph illustrating the quantity of GDNF present in the cell in the differentiated human MSC. Production of GDNF was assayed in cell extracts of the differentiated hBMSc by ELISA. Results are the mean ± S.D. of three independent experiments. Differences where $p < 0.05$ (*) were significant when compared with controls. Differentiated cells were incubated with cabergoline (130pg/ml) and IL-1β (100pg/ml) 24 hours following initiation of differentiation for 48 hours.

FIGs. 8A-D are bar graphs illustrating the quantity of GDNF, BDNF and NGF secreted by the differentiated human MSCs. Figure 8A illustrates the effect of a 48 hour incubation of cabergoline (130pg/ml) and IL-1β (100pg/ml) on the secretion of GDNF. Culture media was collected following 72h of differentiation and analyzed using ELISA. Results are the mean ± S.D. of three independent experiments. Differences where $p < 0.05$ (*) were significant when compared with controls. Differentiated cell media was also assayed for GDNF (Figure 8B) and NGF (Figure 8C). (Donor #14-2 , #8-10 or # H1-2-7. The blue bars depict secretion following differentiation and the red bars depict secretion prior to differentiation. Figure 8D illustrates the effect of a 48 hour differentiation on BDNF secretion in MSCs from three different donors.

FIG. 9 is a bar graph illustrating the expression of the glutamate transporters GLAST and GLT-1 following differentiation of MSCs. The expression of these glutamate transporters was evaluated by Real Time-PCR performed on total RNA extracted from hBMSc grown in serum free medium and astrocyte differentiated hBMSc. Primers for GLAST were SEQ ID NOs: 7 and 8 and primers for GLT-1 were SEQ ID NOs: 13 and 14. Samples were also used in PCR amplification of the GAPDH allowing a quantitative analysis of PCR products expressed in arbitrary units (mean ± SEM from triplicate measures performed on independent cultures). Statistical analysis was performed by one-way anova followed by the Newman–Keul's test for multiple comparisons. *($p < 0.05$) denote significant differences from cells maintained in the serum free medium.

FIG. 10 is a bar graph illustrating the functional activity of glutamate transporters prior to and following astocyte differentiation of hBMSCs. The [^{3}H]d-aspartate uptake (20 nm) was measured in hBMSCs grown in differentiation medium compared to cells grown in serum free medium. Data shown are mean ± SEM from triplicate measures performed on three independent cultures. Statistical analysis was performed by one-way anova followed by the Newman–Keul's test for multiple comparisons ($p < 0.05$).

FIG. 11 is a line graph illustrating the improvement in behaviour of 6-OHDA lesioned rats transplanted with astrocyte differentiated hBMSc. Rats were transplanted with $5X10^5$ cells injected into the ipsilateral striatum 6 week post lesion. Turning behaviour induced by apomorphine (0.15 mg/kg, s.c.) was recorded for a mean number of turns per 60 minutes. Transplanted rats exhibit a marked ($p<0.05$) decrease in rotations 75 days post transplantation.

FIG. 12 is a bar graph illustrating the improvement in rotarod assay of 6-OHDA lesioned rats transplanted with astrocyte differentiated hBMSc. Rotarod performance (seconds on the rod) was observed 95 days post-grafting in the same rats. Transplanted rats exhibit a marked ($p<0.05$) improvement (multiple comparisons tests after Friedman anova, $P<0.05$).

FIG. 13 is a bar graph illustrating the improvement in delicate motor function of 6-OHDA lesioned rats transplanted with astrocyte differentiated hBMSc. A sunflower seed eating test was performed 100 days post transplantation. Astrocyte transplanted rats opened and ate the seeds during a 5 minute period much faster as compared to saline treated rats. Results are mean ±SEM of two tests performed in two following days.

FIGs. 14A-E are confocal microscopy images on cells of 6-OHDA lesioned rats transplanted with astrocyte differentiated hBMSc. At 110 days post transplantation the animals were sacrificed and histology studies were performed. Figure 14A illustrates DAPI staining. Figure 14B illustrates human nuclear antigen staining. Figure 14C illustrates GFAP staining. Figure 14D is a merge of all three stainings. Immunostaining and confocal microscopy study revealed that up to 25 % of the cells that were positive for human antigen were also positive for GFAP.

FIG. 15 is a line graph illustrating that astrocyte differentiated BMSCs transplanted into the gastrocnemius of ALS mice delay the disease onset and improve their motor performance on rotarod ($n=8$, $p<0.05$).

FIG. 16 is a line graph illustrating that astrocyte differentiated BMSC transplanted into the gastrocnemius of ALS mice delay the body weight decline.

FIGs. 17A-C are confocal microscopy images on cells of ALS mice transplanted with astrocyte differentiated hBMSc illustrating that the astrocyte differentiated BMSC transplanted into the gastrocnemius of ALS mice survive 110 days post transplantation.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates to cells and populations thereof which can be transplanted into a patient in order to treat a myriad of neurodegenerative diseases.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Neurotrophic factors (NTFs) are secreted proteins that regulate the survival, functional maintenance and phenotypic development of neuronal cells. Alterations in NTF levels are involved in triggering programmed cell-death in neurons and thus contribute to the pathogenesis of Parkinson's and other neurodegenerative diseases.

One of the most potent NTF for dopaminergic neurons is called glial cell line-derived neurotrophic factor (GDNF). It is known to promote the survival of the dopaminergic neurons in the substantia nigra, promote neurite outgrowth, increase cell body size and also raise levels of TH.

However, direct use of neurotrophic factors in general, and GDNF in particular, is prohibited as they do not pass the blood-brain barrier and do not distribute properly following systemic injection. Therefore, other strategies must be developed in order to take advantage of their therapeutic properties.

Whilst reducing the present invention to practice, the present inventors have found that under specific culturing conditions mesenchymal stem cells (MSCs) may be differentiated into cells having an astrocytic phenotype capable of secreting neurotrophic factors. This outcome was shown to be both donor and passage-independent. Accordingly, the present inventors have shown that such differentiated MSCs can be used to treat patients with neurodegenerative diseases following transplantation.

The present inventors have shown that MSCs differentiated according to a novel two-step protocol represent an astrocyte like shape (Figures 1A-B and 2A-D) accompanied by the presence of astrocyte markers (Figures 3A-B and 4A-B). These astrocyte-like cells were shown to express (Figure 5) and secrete (Figures 8A-D) significant levels of GDNF, BDNF and NGF. Moreover the NTF production was further up-regulated by cabergoline a D2-receptor agonist and IL-1 (Figure 7 and Figure 8A). In addition, the astrocyte-like cells possessed glutamate-clearance machinery (Figure 10).

Following transplantation into the striatum of 6-OHDA lesioned rat (a rat model for Parkinson's) and into the foot muscles of ALS transgenic mice, the cells survived and improved behavioral deficits examined by a rotor rod test, and apomorphine induced rotational behavior (Figures 11-17A-C).

Thus, according to one aspect of the present invention there is provided a method of generating astrocyte-like cells, comprising incubating mesenchymal stem cells in a differentiating medium comprising platelet derived growth factor (PDGF) and human neuregulin 1-β1, thereby generating astrocyte-like cells.

As used herein the phrase "astrocyte-like cells" refers to cells comprising at least one astrocytic phenotype which allows same to in vivo mediate an astrocytic activity, i.e., support of neurons.

Such phenotypes are further described hereinbelow.

The term "mesenchymal stem cell" or "MSC" is used interchangeably for adult cells which are not terminally differentiated, which can divide to yield cells that are either stem cells, or which, irreversibly differentiate to give rise to cells of a mesenchymal cell lineage. The mesenchymal stem cells of the present invention may be of a syngeneic or allogeneic source, although the first is preferred.

According to a preferred embodiment of this aspect of the present invention the mesenchymal stem cells are not genetically manipulated (i.e. transformed with an expression construct) to generate the cells and cell populations described herein.

It will be appreciated that the cells of the present invention may be derived from any stem cell, although preferably not ES cells.

Mesenchymal stem cells may be isolated from various tissues including but not limited to bone marrow, peripheral blood, blood, placenta and adipose tissue. A method of isolating mesenchymal stem cells from peripheral blood is described by Kassis et al [Bone Marrow Transplant. 2006 May;37(10):967-76]. A method of isolating mesenchymal stem cells from placental tissue is described by Zhang et al [Chinese Medical Journal, 2004, 117 (6):882-887]. Methods of isolating and culturing adipose tissue, placental and cord blood mesenchymal stem cells are described by Kern et al [Stem Cells, 2006;24:1294-1301].

According to a preferred embodiment of this aspect of the present invention, the mesenchymal stem cells are human.

Bone marrow can be isolated from the iliac crest of an individual by aspiration. Low-density BM mononuclear cells (BMMNC) may be separated by a FICOL-PAGUE density gradient. In order to obtain mesenchymal stem cells, a cell population comprising the mesenchymal stem cells (e.g. BMMNC) may be cultured in a proliferating medium capable of maintaining and/or expanding the cells. According to one embodiment the populations are plated on polystyrene plastic surfaces (e.g. in a flask) and mesenchymal stem cells are isolated by removing non-adherent cells. Alternatively mesenchymal stem cell may be isolated by FACS using mesenchymal stem cell markers (see Table 1, hereinbelow).

Preferably the MSCs are at least 50 % purified, more preferably at least 75 % purified and even more preferably at least 90 % purified.

Following isolation the cells are typically expanded by culturing in a proliferation medium capable of maintaining and/or expanding the isolated cells *ex vivo* as described in Example 1 hereinbelow. The proliferation medium may be DMEM, alpha-MEM or DMEM/F12. Preferably, the proliferation medium is DMEM. Preferably, the proliferation medium further comprises SPN, L-glutamine and a serum (such as fetal calf serum or horse serum) such as described in Example 1 of the Examples section which follows.

Differentiation to astrocyte-like cells can be effected by incubating the MSCs in differentiating media such as those described in U.S. Pat. No. 6,528,245 and by Sanchez-Ramos *et al.* (2000); Woodburry *et al.* (2000); Woodburry *et al.* (J. Neurisci. Res. 96:908-917, 2001); Black and Woodbury (Blood Cells Mol. Dis. 27:632-635, 2001); Deng *et al.* (2001), Kohyama *et al.* (2001), Reyes and Verfatile (Ann. N. Y. Acad. Sci. 938:231-235, 2001) and Jiang *et al.* (Nature 418:47-49, 2002).

BMSc are preferably incubated in an "additional medium" for at least 24 hours, preferably 48 hours, prior to their incubation in a "differentiation medium". Incubation in "differentiation medium" extends for at least 24 hours, preferably at least 48 hours.

The differentiating media (including the additional differentiating medium) may be DMEM or DMEM/F12, preferably DMEM. A suitable "additional medium" may be any growth medium capable of predisposing the cells to astrocyte-like differentiation, such as a growth medium supplemented with epidermal growth factor hEGF (e.g. 20 ng/ml) and/or basic fibroblast growth factor (e.g. 20 ng/ml). Preferably, the additional medium also comprises N2 supplement (insulin, progesterone, putrescin, selenium and transferrin)

The differentiating medium of the present invention preferably comprises platelet derived growth factor (e.g. 5 ng/ml) and human neuregulin 1-β1 (e.g. 50 ng/ml). The "differentiating medium" preferably includes differentiating agents such as IL-1β and/or dbcAMP.

Preferably the differentiating media further comprise SPN, L-glutamine, a supplement (such as N2 or B27), antibiotic (e.g. IBMX) and a serum (such as fetal calf serum, fetal bovine serum or horse serum).

According to another aspect of the present invention, the mesenchymal stem cells are incubated in a medium comprising at least one differentiating agent in order to generate the astrocyte-like cells of the present invention. Examples of differentiating agents include, but are not limited to platelet derived growth factor (PDGF), human neuregulin 1-β1, FGF2, EGF, N2 supplement, IBMX and cAMP.

The differentiating media (including the additional differentiating medium) may also comprise other agents such as neurotrophic factors (e.g. BDNF, CNTF, GDNF, NTN, NT3 or LIF), hormones, growth factors (e.g. GGF2, TGF-β3, TGF-α, FGF-8 and bFGF), vitamins, hormones e.g., insulin, progesterone and other factors

such as sonic hedgehog, bone morphogenetic proteins, forskolin, retinoic acid, ascorbic acid, putrescin, selenium and transferrin.

Exemplary differentiating media are described in Example 1 hereinbelow.

Cell populations obtained according to the methods describe herein are typically non-homogeneous.

Thus, according to another aspect of the present invention there is provided an isolated population of human cells wherein:

(i) at least N % of the cells comprise at least one astrocytic phenotype;

(ii) at least M % of the cells comprise at least one mesenchymal stem cell phenotype, the mesenchymal stem cell phenotype is not an astrocytic phenotype; and(iii)at least one of the human cells comprises both the at least one astrocytic phenotype and the at least one mesenchymal stem cell phenotype; where M and N are each independently selected between 1 and 99.

The term "isolated" as used herein refers to a population of cells that has been removed from its *in-vivo* location (e.g. bone marrow, neural tissue). Preferably the isolated cell population is substantially free from other substances (e.g., other cells) that are present in its *in-vivo* location.

As used herein, the phrase "astrocytic phenotype" refers to a structural and/or functional parameter typical (e.g. unique) to an astrocyte which may be used to distinguish between the differentiated MSCs of the present invention and non-differentiated MSCs. The astrocytic phenotype may comprise a single or a number of features which may be used to distinguish between the differentiated MSCs of the present invention and non-differentiated MSCs.

It will be appreciated that the functional parameters may overlap with the structural parameter e.g., presence of secretory vesicles.

Preferably the functional astrocytic phenotype comprises the ability to express a neurotrophic factor at a level at least 2 times greater than a basal expression of the neurotrophic factor in a non-differentiated human mesenchymal stem cell.

As used herein the term "express" refers to the synthesis and/or secretion of the above-mentioned neurotrophic factor. Since the neurotrophic factor elicits its effects outside the cell, preferably the secretion of the neurotrophic factor is increased in the cells of the populations of the present invention. According to a preferred embodiment the secretion is increased at least 2 fold compared to the amount of

neurotrophic factor that is secreted in non-differentiated human mesenchymal stem cells and even more preferably 5 fold.

As used herein, the phrase "neurotrophic factor" refers to a cell factor that acts on the cerebral nervous system comprising growth, differentiation, functional maintenance and/or survival effects on neurons. Examples of neurotrophic factors include, but are not limited to, glial derived neurotrophic factor (GDNF), GenBank accession nos. L19063, L15306; nerve growth factor (NGF), GenBank accession no. CAA37703; brain-derived neurotrophic factor (BDNF), GenBank accession no CAA62632; neurotrophin-3 (NT-3), GenBank Accession No. M37763; neurotrophin-4/5; Neurturin (NTN), GenBank Accession No. NP_004549; Neurotrophin-4, GenBank Accession No. M86528; Persephin, GenBank accession no. AAC39640; brain derived neurotrophic factor, (BDNF), GenBank accession no. CAA42761; artemin (ART), GenBank accession no. AAD13110; ciliary neurotrophic factor (CNTF), GenBank accession no. NP_000605; insulin growth factor-I (IGF-1), GenBank accession no. NP_000609; and Neublastin GenBank accession no. AAD21075.

A further example of a functional astrocytic phenotype is the enhancement of expression and/or secretion of a neurotrophic factor following addition of IL-1beta and cabergoline.

Astrocytes play an important role in the maintenance of low extra-cellular glutamate concentration by clearance of glutamate via high-affinity glutamate transporters. Therefore, another functional astrocytic phenotype of the cells of the populations of the present invention may be an increased activity of glutamate transporters. The activity of such glutamate transporters may be analyzed by measuring labeled aspartate (e.g. [^{3}H]-d-aspartate uptake from the culture medium of the cells.

As mentioned hereinabove a percentage of the cells of the cell populations of the present invention may additionally or alternatively comprise a structural astrocytic phenotype.

Examples of structural astrocytic phenotypes include a cell size, a cell shape, an organelle size and an organelle number. Thus, astrocytic structural phenotypes include a round nucleus, a "star shaped" body and many long processes that end as vascular foot plates on the small blood vessels of the CNS (See Figures 1A-B and 2A-D). Further examples of structural astrocytic phenotypes may be found in the

following materials: Reynolds and Weiss, Science (1992) 255:1707-1710; Reynolds, Tetzlaff, and Weiss, J. Neurosci (1992) 12:4565-4574; and Kandel, et al., Principles of Neuroscience, third ed. (1991), Appleton & Lange, Norwalk, Conn. These structural phenotypes may be analyzed using microscopic techniques (e.g. scanning electro microscopy). Antibodies or dyes may be used to highlight distinguishing features in order to aid in the analysis.

A structural astrocytic phenotype may also comprise expression of an astrocyte marker.

As used herein the phrase "astrocyte marker" refers to a polypeptide which is either selectively or non-selectively expressed in an astrocyte. The astrocyte marker may be expressed on the cell surface or internally. Examples of astrocyte markers include S100 beta, glial fibrillary acidic protein (GFAP), glutamine sythetase, GLAST and GLT1.

As mentioned hereinabove, a percentage of cells of the cell populations comprise at least one mesenchymal stem cell phenotype which is not present in typical astrocytic cells. Such stem cell phenotypes are typically structural. For example, the cells of the present invention may show a morphology similar to that of mesenchymal stem cells (a spindle-like morphology). Alternatively or additionally the cells of the present invention may express a marker (e.g. surface marker) typical to mesenchymal stem cells but atypical to native astrocytic cells. Examples of mesenchymal stem cell surface markers include but are not limited to CD105+, CD29+, CD44+, CD90+, CD34-, CD45-, CD19-, CD5-, CD20-, CD11B- and FMC7-. Other mesenchymal stem cell markers include but are not limited to tyrosine hydroxylase, nestin and H-NF.

The cell populations of the present invention also include cells which display both an astrocytic phenotype and a mesenchymal stem cell phenotype. The mesenchymal stem cell phenotype is preferably not as astrocytic phenotype. Thus, as illustrated in Table 3, cells of the cell populations of the present invention were shown to express the mesenchymal stem cell markers, tyrosine hydroxylase, CD90 and H-NF, three markers which are known not be expressed in astrocytes.

Preferably, when cells comprise both the astrocytic and mesenchymal stem cell phenotypes described hereinabove, their astrocytic phenotype is unique to astrocytes. The cells may comprise a single astrocytic phenotype unique to astrocytes

(e.g. star-shaped morphology) or a combination of non-unique astrocytic phenotypes which in combination represent a phenotype unique to astrocytes.

According to one embodiment of the present invention, the astrocytic phenotype of any of the cells of the populations of the present invention is as close as possible to native astrocytes. Thus, as illustrated in the Examples section below, the cells differentiated according to the method of the present invention represent an astrocyte like shape (Figures 1A-B and 2A-D), are accompanied by the presence of astrocyte markers (Figures 3A-B and 4A-B); express (Figure 5) and secrete (Figures 8A-D) significant levels of GDNF, BDNF and NGF; comprise NTF production which is further up-regulated by cabergoline a D2-receptor agonist and IL-1 (Figure 7 and Figure 8A); and possess glutamate-clearance machinery (Figure 10).

The percentage of cells which comprise an astrocytic phenotype may be raised or lowered according to the intended needs. Thus for example, the cell populations may be enriched for cells with a particular astrocytic phenotype (e.g. expression of GDNF). This may be effected by FACS using an antibody specific for an astrocyte cell marker. Examples of such astrocytic markers are described hereinabove. If the cell marker is an internal marker, preferably the FACS analysis comprises antibodies or fragments thereof which may easily penetrate a cell and may easily be washed out of the cell following detection. The FACS process may be repeated a number of times using the same or different markers depending on the degree of enrichment and the cell phenotype required as the end product.

According to another embodiment of this aspect of the present invention the cell populations may be enriched for cells comprising both an astrocytic phenotype and a mesenchymal stem cell phenotype such that a homogeneous population of cells are generated.

Thus, according to yet a further aspect of the present invention there is provided an isolated human cell comprising at least one astrocytic phenotype and at least one mesenchymal stem cell phenotype, wherein the mesenchymal stem cell phenotype is not an astrocytic phenotype.

Once differentiated and optionally isolated, the cells may be tested (in culture) for their astrocytic phenotype (e.g. ability to secrete a functional neurotrophic factor). The cultures may be comparatively analyzed for an astrocytic phenotype, using biochemical analytical methods such as immunoassays, Western blot and Real-time

PCR as described in Examples 1 of the Examples section which follows, or by enzyme activity bioassays.

According to the astrocytic phenotype, the cells and cell populations of the present invention may be used to treat a particular disease or disorder. The cell populations may be used directly following differentiation or may be enriched for a particular astrocytic phenotype as described hereinabove. As summarized in Table 1 hereinbelow, certain neurotrophic factors or set of neurotrophic factors have been shown to be particularly beneficial for treating a particular disease. For example, cells of the present invention which secrete NGF, BDNF, FGF and GDNF would be particularly suitable for treating Parkinson's.

Table 1

Disease	***Astrocytic phenotype***	***REF***
Parkinsons	NGF, BDNF, FGF, GDNF	Walker DG, et al. Brain Res 1998; 794: 181-7. Lorigados L, et al. Rev Neurol 1998; 26: 744-8. Mogi M, et al. Neurosci Lett 1994; 180: 147-50. Howells DW, et al. Exp Neurol 2000; 166: 127-35. Beck KD, et al. Nature 1995; 373: 339-41. Tomac A, et al. Nature 1995; 373: 335-9. Gash DM, et al. Nature 1996; 380: 252-5. Choi-Lundberg DL, Science 1997; 275: 838-41. Bozzi Y, Borrelli E. Eur J Neurosci 1999; 11: 1275-84. Chauhan NB, et al Soc Neurosci Abstr 1998; 24: 1465. Chauhan NB, et al, Neurology 1999; 52:A212-213.
Epilepsy	BDNF, NGF, NT-3, glutamate transporter	G.W. Mathern, Mol. Chem. Neuropathol. 30 1–2 (1997), pp. 53–76. Lucia Tapia-Arancibia et al. Frontiers in Neuroendocrinology 2004 Jul;25(2):77-107. RYUTA KOYAMA and YUJI IKEGAYA;NEUROSCIENCE UPDATE 2005 Aug;11(4):282-7. Gerald Seifert, et al., Nature Reviews Neuroscience 7, 194-206 (March 2006).
ALS	NT3, IGF1, BDNF, glutamate transporter	Luis H. Et al., Brain Research Reviews 2004 Dec;47(1-3):263-74. Bradley WG. Ann Neurol 1995; 38: 971. Haase G, et al. Nat Med 1997; 3:429-36. Arakawa Y, J Neurosci 1990; 10: 3507-15.
neuropathy	NGF	G. Sobue, M. et al. Neurochem. Res. 23 6 (1998), pp. 821–829.
Drug and alcohol addiction	GDNF	Ron D, Janak PH. Rev Neurosci. 2005;16(4):277-85.
Brain injury	Ability of cells to respond to IL-1	Nancy Rothwell; Brain, Behavior, and Immunity. 2003 Jun;17(3):152-7 .
Alzheimers	NGF BDNF	Crutcher KA, et al. J Neurosci 1993; 6: 2540-50. Scott SA, et al. Nerve growth factor in Alzheimer's disease: increased levels throughout the brain coupled with declines in nucleus basalis. J Neurosci 1995; 15: 6213-21. Peng S, et al. J Neuropathol Exp Neurol 2004; 63: 641-9. Murer MG, et al. Neuroscience 1999; 88: 1015-32.
Huntingdon's	BDNF, NT-3, or NT-4/5	Martinez-Serrano A, Bjorklund A. Trends Neurosci 1997; 20: 530-8.

		Perez-Navarro E, et al. J Neurochem 2000; 75: 2190-9. Perez-Navarro E, et al. Neuroscience 1999; 91: 1257-64.
Schizophrenia	NGF, NT-3, BDNF	Gal Shoval, Abraham Weizmana; Eur Neuropsychopharmacol. 2005 May;15(3):319-29. Levi-Montalcini, R., 1987. Biosci. Rep. 7, 681– 699. Hattori, M., Nanko, S., 1995. Biochem. Biophys. Res. Commun. 209, 513– 518. Virgos, C., 2001, Schizophr. Res. 49, 65–71.
Optic nerve	CNTF	Paul A. Sieving, et al.,Proc Natl Acad Sci U S A. 2006 Mar 7;103(10):3896-901.
Stroke	FGF,BDNF	Wu D; Neuro Rx. 2005 Jan;2(1):120-8.

It has been proposed that astrocyte cells may reduce the oxidative stress in neurons by metabolizing dopamine, as they express monoamine oxidase-B and catechol-O-methyl-transferase Additionally, it has been proposed that astrocyte cells may be capable of preventing NO generated neurotoxicity by a glutathione dependent mechanism (Chen et al. 2004, Curr Drug Targets. 2005 Nov;6(7):821-33). Accordingly, cells of the present invention which comprise a scavenging function and/or express dopamine metabolizing enzymes may also be suitable for treating Parkinson's.

Owing to insufficient clearance or decrease of the glutamate transporters glutamate excitotoxicity has been suggested as a causative factor for ALS [Bendotti 2001, et al, J Neurochem, 79(4):737-746, 2001]. Thus cells of the present invention which show an elevated glutamate transporter activity may also be suitable for treating ALS.

Thus, according to another aspect of the present invention there is provided a method of treating a CNS disease or disorder.

As used herein, the phrase "CNS disease" refers to any disorder, disease or condition of the central nervous system which may be treated with the cells of the present invention.

Accordingly, these cells can be used for preparing a medicament (interchangeably referred to as pharmaceutical composition), whereby such a medicament is formulated for treating a CNS disease or disorder.

Representative examples of CNS diseases or disorders that can be beneficially treated with the cells described herein include, but are not limited to, a pain disorder, a motion disorder, a dissociative disorder, a mood disorder, an affective disorder, a neurodegenerative disease or disorder and a convulsive disorder.

More specific examples of such conditions include, but are not limited to, Parkinson's, ALS, Multiple Sclerosis, Huntingdon's disease, autoimmune

encephalomyelitis, diabetic neuropathy, glaucatomus neuropathy, macular degeneration, action tremors and tardive dyskinesia, panic, anxiety, depression, alcoholism, insomnia, manic behavior, Alzheimer's and epilepsy.

In any of the methods described herein the cells may be obtained from any autologous or non-autologous (i.e., allogeneic or xenogeneic) human donor. For example, cells may be isolated from a human cadaver or a donor subject.

The cells of the present invention can be administered to the treated individual using a variety of transplantation approaches, the nature of which depends on the site of implantation.

The term or phrase "transplantation", "cell replacement" or "grafting" are used interchangeably herein and refer to the introduction of the cells of the present invention to target tissue. The cells can be derived from the recipient or from an allogeneic or xenogeneic donor.

The cells can be grafted into the central nervous system or into the ventricular cavities or subdurally onto the surface of a host brain. Conditions for successful transplantation include: (i) viability of the implant; (ii) retention of the graft at the site of transplantation; and (iii) minimum amount of pathological reaction at the site of transplantation. Methods for transplanting various nerve tissues, for example embryonic brain tissue, into host brains have been described in: "Neural grafting in the mammalian CNS", Bjorklund and Stenevi, eds. (1985); Freed et al., 2001; Olanow et al., 2003). These procedures include intraparenchymal transplantation, i.e. within the host brain (as compared to outside the brain or extraparenchymal transplantation) achieved by injection or deposition of tissue within the host brain so as to be opposed to the brain parenchyma at the time of transplantation.

Intraparenchymal transplantation can be effected using two approaches: (i) injection of cells into the host brain parenchyma or (ii) preparing a cavity by surgical means to expose the host brain parenchyma and then depositing the graft into the cavity. Both methods provide parenchymal deposition between the graft and host brain tissue at the time of grafting, and both facilitate anatomical integration between the graft and host brain tissue. This is of importance if it is required that the graft becomes an integral part of the host brain and survives for the life of the host.

Alternatively, the graft may be placed in a ventricle, e.g. a cerebral ventricle or subdurally, i.e. on the surface of the host brain where it is separated from the host brain parenchyma by the intervening pia mater or arachnoid and pia mater. Grafting to

the ventricle may be accomplished by injection of the donor cells or by growing the cells in a substrate such as 3% collagen to form a plug of solid tissue which may then be implanted into the ventricle to prevent dislocation of the graft. For subdural grafting, the cells may be injected around the surface of the brain after making a slit in the dura. Injections into selected regions of the host brain may be made by drilling a hole and piercing the dura to permit the needle of a microsyringe to be inserted. The microsyringe is preferably mounted in a stereotaxic frame and three dimensional stereotaxic coordinates are selected for placing the needle into the desired location of the brain or spinal cord. The cells may also be introduced into the putamen, nucleus basalis, hippocampus cortex, striatum, substantia nigra or caudate regions of the brain, as well as the spinal cord.

The cells may also be transplanted to a healthy region of the tissue. In some cases the exact location of the damaged tissue area may be unknown and the cells may be inadvertently transplanted to a healthy region. In other cases, it may be preferable to administer the cells to a healthy region, thereby avoiding any further damage to that region. Whatever the case, following transplantation, the cells preferably migrate to the damaged area.

For transplanting, the cell suspension is drawn up into the syringe and administered to anesthetized transplantation recipients. Multiple injections may be made using this procedure.

The cellular suspension procedure thus permits grafting of the cells to any predetermined site in the brain or spinal cord, is relatively non-traumatic, allows multiple grafting simultaneously in several different sites or the same site using the same cell suspension, and permits mixtures of cells from different anatomical regions. Multiple grafts may consist of a mixture of cell types, and/or a mixture of transgenes inserted into the cells. Preferably from approximately 10^4 to approximately 10^8 cells are introduced per graft.

For transplantation into cavities, which may be preferred for spinal cord grafting, tissue is removed from regions close to the external surface of the central nerve system (CNS) to form a transplantation cavity, for example as described by Stenevi *et al.* (Brain Res. 114:1-20., 1976), by removing bone overlying the brain and stopping bleeding with a material such a gelfoam. Suction may be used to create the cavity. The graft is then placed in the cavity. More than one transplant may be placed in the same cavity using injection of cells or solid tissue implants. Preferably, the site

of implantation is dictated by the CNS disorder being treated and the astrocytic phenotype comprised in the cell (e.g. particular neurotrophic factor being secreted) by the cells of the present invention. For example, cells secreting GDNF are preferably implanted in the sabstantia nigra of a Parkinson's patient.

Since non-autologous cells are likely to induce an immune reaction when administered to the body several approaches have been developed to reduce the likelihood of rejection of non-autologous cells. These include either suppressing the recipient immune system or encapsulating the non-autologous cells in immunoisolating, semipermeable membranes before transplantation.

Encapsulation techniques are generally classified as microencapsulation, involving small spherical vehicles and macroencapsulation, involving larger flat-sheet and hollow-fiber membranes (Uludag, H. et al. Technology of mammalian cell encapsulation. Adv Drug Deliv Rev. 2000; 42: 29-64).

Methods of preparing microcapsules are known in the arts and include for example those disclosed by Lu MZ, et al., Cell encapsulation with alginate and alpha-phenoxycinnamylidene-acetylated poly(allylamine). Biotechnol Bioeng. 2000, 70: 479-83, Chang TM and Prakash S. Procedures for microencapsulation of enzymes, cells and genetically engineered microorganisms. Mol Biotechnol. 2001, 17: 249-60, and Lu MZ, et al., A novel cell encapsulation method using photosensitive poly(allylamine alpha-cyanocinnamylideneacetate). J Microencapsul. 2000, 17: 245-51.

For example, microcapsules are prepared by complexing modified collagen with a ter-polymer shell of 2-hydroxyethyl methylacrylate (HEMA), methacrylic acid (MAA) and methyl methacrylate (MMA), resulting in a capsule thickness of 2-5 μm. Such microcapsules can be further encapsulated with additional 2-5 μm ter-polymer shells in order to impart a negatively charged smooth surface and to minimize plasma protein absorption (Chia, S.M. et al. Multi-layered microcapsules for cell encapsulation Biomaterials. 2002 23: 849-56).

Other microcapsules are based on alginate, a marine polysaccharide (Sambanis, A. Encapsulated islets in diabetes treatment. Diabetes Technol. Ther. 2003, 5: 665-8) or its derivatives. For example, microcapsules can be prepared by the polyelectrolyte complexation between the polyanions sodium alginate and sodium

cellulose sulphate with the polycation poly(methylene-co-guanidine) hydrochloride in the presence of calcium chloride.

It will be appreciated that cell encapsulation is improved when smaller capsules are used. Thus, the quality control, mechanical stability, diffusion properties, and *in vitro* activities of encapsulated cells improved when the capsule size was reduced from 1 mm to 400 μm (Canaple L. et al., Improving cell encapsulation through size control. J Biomater Sci Polym Ed. 2002;13:783-96). Moreover, nanoporous biocapsules with well-controlled pore size as small as 7 nm, tailored surface chemistries and precise microarchitectures were found to successfully immunoisolate microenvironments for cells (Williams D. Small is beautiful: microparticle and nanoparticle technology in medical devices. Med Device Technol. 1999, 10: 6-9; Desai, T.A. Microfabrication technology for pancreatic cell encapsulation. Expert Opin Biol Ther. 2002, 2: 633-46).

Examples of immunosuppressive agents include, but are not limited to, methotrexate, cyclophosphamide, cyclosporine, cyclosporin A, chloroquine, hydroxychloroquine, sulfasalazine (sulphasalazopyrine), gold salts, D-penicillamine, leflunomide, azathioprine, anakinra, infliximab (REMICADE.sup.R), etanercept, TNF.alpha. blockers, a biological agent that targets an inflammatory cytokine, and Non-Steroidal Anti-Inflammatory Drug (NSAIDs). Examples of NSAIDs include, but are not limited to acetyl salicylic acid, choline magnesium salicylate, diflunisal, magnesium salicylate, salsalate, sodium salicylate, diclofenac, etodolac, fenoprofen, flurbiprofen, indomethacin, ketoprofen, ketorolac, meclofenamate, naproxen, nabumetone, phenylbutazone, piroxicam, sulindac, tolmetin, acetaminophen, ibuprofen, Cox-2 inhibitors and tramadol.

In any of the methods described herein, the cells can be administered either *per se* or, preferably as a part of a pharmaceutical composition that further comprises a pharmaceutically acceptable carrier.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the chemical conjugates described herein, with other chemical components such as pharmaceutically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to a subject.

Hereinafter, the term "pharmaceutically acceptable carrier" refers to a carrier or a diluent that does not cause significant irritation to a subject and does not abrogate the

biological activity and properties of the administered compound. Examples, without limitations, of carriers are propylene glycol, saline, emulsions and mixtures of organic solvents with water.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

According to a preferred embodiment of the present invention, the pharmaceutical carrier is an aqueous solution of saline.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration include direct administration into the tissue or organ of interest. Thus, for example the cells may be administered directly into the brain as described hereinabove or directly into the muscle as described in Example 3 hereinbelow.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from *in vitro* and cell culture assays. Preferably, a dose is formulated in an animal model to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures *in vitro*, in cell cultures or experimental animals. For example, 6-OHDA-lesioned mice may be used as animal models of Parkinson's. In addition, a sunflower test may be used to test improvement in delicate motor function by challenging the animals to open sunflowers seeds during a particular time period.

Transgenic mice may be used as a model for Huntingdon's disease which comprise increased numbers of CAG repeats have intranuclear inclusions of huntingtin and ubiquitin in neurons of the striatum and cerebral cortex but not in the brain stem, thalamus, or spinal cord, matching closely the sites of neuronal cell loss in the disease.

Transgenic mice may be used as a model for ALS disease which comprise SOD-1 mutations.

The septohippocampal pathway, transected unilaterally by cutting the fimbria, mimics the cholinergic deficit of the septohippocampal pathway loss in Alzheimers disease. Accordingly animal models comprising this lesion may be used to test the cells of the present invention for treating Alzheimers.

Survival and rotational behavior (e.g. on a rotarod) of the animals may be analyzed (as in Examples 2 and 3) following administration of the cells of the present invention.

The data obtained from these *in vitro* and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition, (see e.g., Fingl, *et al.*, 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1). For example, Parkinson's patient can be monitored symptomatically for improved motor functions indicating positive response to treatment.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer.

Dosage amount and interval may be adjusted individually to levels of the active ingredient which are sufficient to effectively regulate the neurotransmitter synthesis by the implanted cells. Dosages necessary to achieve the desired effect will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the individual being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc. The dosage and timing of administration will be responsive to a careful and continuous monitoring of the individual changing condition. For example, a treated Parkinson's patient will be

administered with an amount of cells which is sufficient to alleviate the symptoms of the disease, based on the monitoring indications.

The cells of the present invention may be co-administered with therapeutic agents useful in treating neurodegenerative disorders, such as gangliosides; antibiotics, neurotransmitters, neurohormones, toxins, neurite promoting molecules; and antimetabolites and precursors of neurotransmitter molecules such as L-DOPA. Additionally, the cells of the present invention may be co-administered with other cells capable of synthesizing a neurotransmitter. Such cells are described in U.S. Pat. Appl. No. 20050265983 to the present inventors.

Following transplantation, the cells of the present invention preferably survive in the diseased area for a period of time (e.g. at least 6 months), such that a therapeutic effect is observed. As described in Example 2, the cells of the present invention were shown to be viable in the 6-OHDA lesioned mouse brain hemisphere at 16 weeks following transplantation and viable in the transgenic mouse model for ALS at 110 days post transplantation.

As used herein the term "about" refers to ± 10 %.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning",

John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1

Characterization of astrocyte-differentiated BMSCs

MATERIALS AND METHODS

Isolation and culture of human BMSC: Bone marrow aspirations (10-30 ml) were collected from the posterior iliac crest of healthy adult human donors with informed consent. Low-density BM mononuclear cells (BMMNC) were separated by

FICOL-PAGUE density gradient (1·077 g/ml) and washed with HBSS. Next, the cells were plated in polystyrene plastic flasks in growth medium. Growth medium consisted of dulbecco's modifed eagle's medium (DMEM) supplemented with 15 % fetal calf serum (FCS), 2 mM L-glutamine, 100 µg/ml streptomycin, 100 U/ml penicillin, 12.5 units/ml nystatin (SPN). The non adherent cells were removed with medium replacement. Following confluence, adherent layers were trypsinized, reseeded, grown to confluence and passaged 2 to 6 times prior to experiments. Growth medium was changed twice a week and cells were maintained at 37 °C in a humidified 5 % CO_2 incubator.

Astrocyte differentiation: To induce astrocyte differentiation, $1X10^6$ cells were cultured in 10 mm dishes. Cells were replaced with pre-differentiation medium for 48 hours (DMEM supplemented with SPN, 2 mM L-glutamine, 20 ng/ml human epidermal growth factor hEGF (R& D, Systems, Minneapolis, MN), 20 ng/ml human basic fibroblast growth factor (hbFGF) and N2 supplement (insulin 5µg/ml, progesterone 20 nM, putrescin 100 µM, selenium 30 nM, transferrin 100 µg/ml). After 48 hours, the pre-differentiation medium was changed to differentiation medium, (DMEM supplemented with SPN, 2 mM L-glutamine, 1 mM dibutyryl cyclic AMP dbcAMP, 0.5 mM isobutylmethylxanthine IBMX (Sigma-Aldrich, St. Louis, Missouri, USA), 5 ng/ml human platelet derived growth factor PDGF (peprotech), and 50 ng/ml human neuregulin 1-β1 NRG1-β1-GGF-2). In some experiments IL-1β (100 pg/ml) or Cabergoline (130pg/ml) were added into the medium 24 hours following initiation of differentiation.

Immunocytochemistry: For immunochemistry analysis, cells were grown on 12 mm round poly-L-lysine coated glass coverslips. At the end of the experiment the medium was removed, cells were fixed with paraformaldehyde 4 % (v/v) for 20 minutes at room temperature and permeabilized thereafter with 0.25 % Triton X-100 (v/v) in 0.1M PBS for 20 minutes. Non-specific binding was blocked by incubating the cells in a 0.1M PBS solution containing 5 % normal goat serum (NGS) and 1 % bovine serum albumin (BSA) (Sigma) for 1 hour at 37 °C. Subsequently, the cells were incubated in a 0.1M PBS solution containing 0.25 % Triton X-100 (v/v), 5 % NGS and 1 % BSA with primary antibodies i.e. rabbit anti-glial fibrillary acidic protein (GFAP) 1:100 (DAKO), rabbit anti-GDNF 1:100 (Santa Cruz Biotechnology Inc., Santa Cruz, California, USA), mouse anti human nuclear h-Nuc 1:30, rabbit

anti-Glutamine synthetase 1:200 (Sigma-Aldrich, St. Louis, Missouri, USA), mouse anti S100β 1:200 (Sigma-Aldrich, St. Louis, Missouri, USA). Secondary antibodies; for GDNF staining goat anti rabbit biotinylated (1:200) was added for 1 hour and subsequently streptavidine-Alexa-488 conjugated goat anti-rabbit IgG antibody 1:200 (Molecular Probes, Eugene, OR, USA). For other staining, Alexa-488 conjugated goat anti-rabbit IgG antibody 1:200 and Rodamine-Rx-conjugated 1:200 (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) were diluted in 0.1M PBS solution containing 0.25 % Triton X-100 (v/v), 5 % NGS and 1 % BSA and were applied for 1 hour at room temperature. Nuclei were stained for 5 minutes with the nuclear dye DAPI 1:200 (Sigma, Aldrich). Following three rinses in PBS, the preparations were mounted in Antifaiding (Sigma, Israel) and examined using a fluorescent microscope coupled to a CCD camera (T.I.L.L. photonics, Martinsried, Germany). Excitation wavelengths (488, 405 and 568 nm for Alexa 488, DAPI and Alexa 568, respectively) were generated using a Xenon lamp coupled to a monochromator (T.I.L.L. photonics, Martinsried, Germany). Digital images were acquired using appropriate filters and combined using the TILLvisION software.

GDNF and BDNF analysis: At the end of the differentiating procedure described above, supernatant was collected from the plate and cells were harvested and counted. The amount of GDNF or BDNF in the cell's lysate and culture supernatants was quantified by using a GDNF or BDNF ImmunoAssay System (Promega) according to the manufacture's protocol. The absorbance at 450 nm was recorded on the Microplate Reader (BioRad Model 550) and the values were used to produce the curve of the GDNF or BDNF standard. The GDNF standard curve was linear between 15.6 and 1,000 pg/ml. ELISA results were calculated according to 10^6 cells per plate.

Cell count assessment: In order to determine the number of positive cells expressing a particular antigen, data were collected from careful examination of 15 fields from three independent cultures per sample. The total number of MSCs in these samples was determined by counting DAPI-stained cell nuclei and results are expressed as percentages of positive cells for either GDNF, glutamine synthetase, or S100β.

Preparation of mRNA: Total RNA was isolated as described by Chomczynski and Sacchi (Anal Biochem 162:156-159, 1987). Cells were disrupted with 200 µl of

solution D (4 M guanidinium thioyanate, 25 mM sodium citrate, 0.5 % sarcosyl, 0.1 M 2-mercaptoethanol). Subsequently, 0.1 vol. of 2 M sodium acetate (pH 4), 1 vol. phenol (saturated) and 0.2 vol. of chloroform: isoamyl alchol (49:1) were added. Samples were allowed to sit at 4 °C for 30 minutes, and then centrifuged at 10,000Xg for 20 minutes at 4 °C. The upper aqueous phase was mixed with 2 vols. of ethanol and the RNA was allowed to precipitate overnight at -20 °C. The final RNA pellets were washed in 75 % ethanol, and air dried. The pellets were dissolved in DEPC-treated water. The amount of RNA was determined spectrophotometrically using the ND-1000 spectrophotometer (Nano-drop). RNA quality was verified by measuring OD260/OD280 ratio. RNA was stored at -80°C until used.

Real-time quantitative reverse transcription polymerase chain reaction: Real-time quantitative PCR of the desired genes was performed in an ABI Prism 7700 sequence detection system (Applied biosystems) using Sybr green PCR master mix (Applied biosystems) and the following primers: GAPDH sense- 5'-CGACAGTCAGCCGCATCTT-3' (SEQ ID NO: 1), GAPDH antisense 5'-CCAATACGACCAAATCCGTTG -3' (SEQ ID NO: 2); GDNF sense 5'-TCAAATATGCCAGAGGATTATCCTG-3' (SEQ ID NO: 3), GDNF antisense 5'-GCCATTTGTTTATCTGGTGACCTT-3' (SEQ ID NO: 4): BDNF sense 5'-AGCTCCGGGTTGGTATACTGG-3' (SEQ ID NO: 5), BDNF antisense 5'-CCTGGTGGAACTTCTTTGCG-3' (SEQ ID NO: 6): GLAST sense 5'-AGAATGAGCTACC GGGAAGTCA-3' (SEQ ID NO: 7): GLAST anti sense 5'-CTAGC GCCG CCATTCCT-3' (SEQ ID NO: 8); NGF sense 5'-CATGCTGGACCCAAGCTCA-3' (SEQ ID NO: 9); NGF anti sense 5'-GACATTACGCTAT GCACCTCAGTG-3' (SEQ ID NO: 10); GFAP sense TAGAGGGCGA GGAGAACCG -3' (SEQ ID NO: 11); GFAP anti sense 5'GTGGCCTTCTGACACAGACTTG -3' (SEQ ID NO: 12); GLT-1 sense 5'-TTGGCTCAGAGGA ACCCAAG-3' (SEQ ID NO: 13), GLT-1 anti sense 5'-CAGGATGACAC CAAA CACCGT-3' (SEQ ID NO: 14); S100 beta sense 5'-GGGTGAGACAAGGAAGAGGATG-3' (SEQ ID NO: 15), S100 beta anti-sense 5'-GCTTGTGCTT GTCTCCCTCC-3' (SEQ ID NO: 16); Glutamine synthetase sense 5'-CGAAGGCCTGCAGAGACC-3' (SEQ ID NO: 17), Glutamine synthetase anti sense 5'-AGGGTATACTCCTGCTCCATGC-3' (SEQ ID NO: 18). GAPDH gene served as a valid reference 'housekeeping' gene for transcription profiling.

For quantitation, real time quantitative PCR (qPCR) was performed in duplicates. qPCR amplification was performed for each sample in separate wells of the same PCR reaction plate, which also contained standard curve for each gene amplified and no template controls (NTC). Optimal experimental parameters (hybridization temperature, elongation time, and primers concentration) were determined for each primer pair. For each gene, verifying a single peak in melting curve analysis assessed the specificity of the PCR product.

The PCR was performed in a total volume of 20 µl containing 1 µl of the above-described cDNA, 1 µl each of the 3' and 5' primers (final concentration of 500 nmol/L each), 10 µl of AbsoluteTM QPCR SYBR® Green ROX Mix and 8 µl of DEPC water.

The amplification protocol was 40 cycles of 95 °C for 15 sec followed by 60 °C for 1 min each. Quantitative calculations of the gene of interest versus GAPDH was performed using the ddCT method, as instructed in the user bulletin #2 ABI prism 7700 sequence detection system (updated 10/2001).

Reverse transcription polymerase chain reaction (RT-PCR): RT-PCR was carried out on 0.5 microgram RNA samples using the 10U enzyme RT-superscript II (Gibco-BRL, MD, USA) in a mixture containing 1.3 µM oligo-dT12-18 (Sigma, USA), 1xBuffer supplied by the manufacturer, 10 mM DTT, 20 µM dNTPs, and RNase inhibitor (RNAguard, amersham pharmacia biotech, UK). Reverse transcription was performed at 42 °C for 2 hours. PCR of the desired genes was performed with specific primers in a mixture containing 2 µl RT, 1Xbuffer (Takara, Japan), 200 µM dNTPs, 1 µM of each primer and 5U units of Taq polymerase (Takara, Japan) followed by 25-30 cycles in Thermocycler: 94 °C for 30 seconds, annealing 55 °C for 30 seconds and DNA extension at 72 °C for 45 seconds. Under these experimental conditions, linearity of the amplification was observed.

RT-PCR was performed on non-differentiated hBMSCs and the differentiated BMSCs according to the method of the present invention. Expression of the genes was compared between the two cell types and to expression in astrocytes (from information obtained from the literature). The primer sequences are detailed in Table 2 hereinbelow.

Table 2

GENE	*5' PRIMER*	*3' PRIMER*	*NCBI*
CD90	ctagtggaccagagccttcg SEQ ID NO: 19	gccctcacacttgaccagtt SEQ ID NO: 20	NM6288
CD34	aaggagcagggagcatac SEQ ID NO: 21	tgcatgtgcagactcctttc SEQ ID NO: 22	NM1773
CD133	gatgcagaacttgacaacg SEQ ID NO: 23	acacagtaagcccaggtagt SEQ ID NO: 24	NM006017
Nestin	ccagaaactcaagcaccac SEQ ID NO: 25	ttttccactccagccatcc SEQ ID NO: 26	X65964
H-NF	aaagcaccaaggactcact SEQ ID NO: 27	ttctcagacttctccaccac SEQ ID NO: 28	NM021076
TH	gaggggaaggccgtgctaaa SEQ ID NO: 29	gaggcgcacgaagtactcca SEQ ID NO: 30	NM000360
NGFR	ctacggctactaccaggatg SEQ ID NO: 31	ctggctatgaggtcttgttc SEQ ID NO: 32	NM002507
GDNF	tcaaatatgccagaggattatcctg SEQ ID NO: 33	gccatttgtttatctggtgacctt SEQ ID NO: 34	NM199231.1
NGF	catgctggacccaagctca SEQ ID NO: 35	gacattacgctatgcacctcagtg SEQ ID NO: 36	NM002506.2
IGF1	tcagctcgctctgtccgtg SEQ ID NO: 37	ttgcgttcttcaaatgtacttcct SEQ ID NO: 38	NM000618
BDNF	agctccgggttggtatactgg SEQ ID NO: 39	cctggtggaacttctttgcg SEQ ID NO: 40	NM170732.3
CNTF	cctgactgctcttacggaatcctat SEQ ID NO: 41	cccatccgcagagtccag SEQ ID NO: 42	NM000614.2
GFAP	tagagggcgaggagaaccg SEQ ID NO: 43	gtggccttctgacacagacttg SEQ ID NO: 44	NM003054
S100b	gggtgagacaaggaagaggatg SEQ ID NO: 45	gcttgtgcttgtctccctcc SEQ ID NO: 46	NM006272.1
NT3 Neurotrophin	gacttcagagaacaataaactcgtgg SEQ ID NO: 47	tgccaattcatgttcttccg SEQ ID NO: 48	NM002527.3
GLAST	agaatgagctaccgggaagtca SEQ ID NO: 49	ctagcgccgccattcct SEQ ID NO: 50	Nm004172.3
GLUL	cgaaggcctgcagagacc SEQ ID NO: 51	agggtatactcctgctccatg SEQ ID NO: 52	NM02065.4
GLT1	ttggctcagaggaacccaag SEQ ID NO: 53	caggatgacaccaaacaccgt SEQ ID NO: 54	D85884
GAPDH	cgacagtcagccgcatctt SEQ ID NO: 55	ccaatacgaccaaatccgttg SEQ ID NO: 56	NM002046

Scanning electron microscopy: The cells were fixed with 2.5 % glutaraldehyde in phosphate buffer (pH 7.2), washed in the same buffer and post fixed with 2 % OsO4. The third step of fixation was performed using a solution of tannic acid and guanidine hydrochloride. The triple-fixed cells were dehydrated in graded alcohol solutions. The alcohol was then exchanged for Freon-112 using graded Freon solutions. The cells were air-dried, gold coated and examined using a Jeol 840 scanning electron microscope.

Glutamate uptake: Functional characterization of glutamate transporters was performed on trypsinized differentiated cells. Transport velocity was estimated by measuring the uptake of [^{3}H]-d-aspartate (20 nM) following a six minute incubation at

37 °C in a buffer containing Na+. The specific activity of the glutamate transporters was expressed as the uptake velocity per the quantity of protein in mg.

RESULTS

hBMSc differentiated according to the method of the present invention acquired a satellite like morphology resembling astrocyte cells whereas the control cells that were grown in serum free medium alone, exhibited a flat fibroblast like morphology characteristic of hBMSc (Figures 1A-B). The differentiated cells were also analyzed using a scanning electro-microscope which confirmed their unique astrocytic satellite morphology (Figures 2A-D).

The astrocyte phenotype of the cells was further verified using immunofluorescence analysis with typical astrocyte markers. As illustrated in Figures 3A-B, the morphological change was accompanied with positive immunostaining for S100 beta, (a subunit of astrocyte Ca+ channels) and glutamine synthase (GS), (a unique astrocyte enzyme that catabolize glutamate). In addition, the cells stained positive for glial fibrillary acidic protein (GFAP) (Figure 4A).

Quantification of the immunocytochemistry staining data revealed that the percentage of the positive cells for the S100 beta, GFAP and GS are in the range of 20-30 %.

To further confirm the expression of astrocyitic markers, real-time PCR analysis was performed. As demonstrated in Figure 4B, the GFAP transcript was up-regulated by 10-20 fold. Similar values were shown in S100 beta and GS transcripts.

Since astrocytes are known for their secretion of neurotrophic factors (NTFs), the mRNA levels of NTFs such as GDNF, NGF and BDNF were analyzed. Real time PCR analysis revealed that astrocyte differentiated hBMSc expressed high quantities of NTFs transcripts as compared to low or absence of these transcripts in MSC grown in serum free medium (Figure 5). Immunostaining with anti-GDNF revealed 30 % of the cultured cells positively stained with the antibody (Figures 6A-B).

An ELISA assay with specific antibodies was used to quantify the production and secretion of NTFs. Measurement of GDNF production was tested in the cell extract (Figure 7) and in the growth medium Figure 8A. Although a basal level of GDNF production was detected in the cultured MSC and neuroblastoma cell line (SH-SY5Y treated with rasagelein), in the culture media GDNF was detected only in the differentiated MSC.

Moreover, a marked enhanced production and secretion of GDNF was detected following addition of IL-1beta and cabergoline, factors which are known to upregulate GDNF. This phenomenon supports the notion that the differentiated cells respond as astrocytes. The levels of secreted GDNF and NGF were compared in several donors and it can be seen that the levels of secretion vary between 69-140pg/10^6 cells for GDNF (Figure 8B) and 356-875pg/10^6 cells for NGF (Figure 8C).

Astrocytes play an important role in the maintenance of low extra-cellular glutamate concentration by clearance of glutamate via high-affinity glutamate transporters and catabolizing it by glutamine synthetase. Therefore, the differentiated cells were analyzed, using RT-PCR analysis for expression of glutamate transporters and glutamine synthetase. A high expression of GLT-1/EAAT-2 transcript was found in astrocyte differentiated hBMSc (Figure 9). The activity of the glutamate transporter was evident by the ^{3}H-glutamate uptake from the culture medium of the differentiated cells but not from the MSC ($p<0.005$) (Figure 10).

A more detailed RT-PCR analysis was performed in order to determine which mesenchymal cell and which astrocytic cell markers were expressed by the cells of the present invention. The expression of the genes was in the two cell types was also compared to expression of the genes in astrocytes (information obtained from the literature). The results are summarized in Table 3 hereinbelow.

Table 3

GENE	*hMSc*	*DIF*	*Astrocyte*
CD90	+	+	-
CD34	-	-	-
CD133	-	-	-
Nestin	++	+	+/+++/-
H-NF	+	+	-
TH	+	+	-
NGFR	+	+	+++
GDNF	+	++	+++
NGF	+	+++	+++
IGF1	+	++	+++
BDNF	+	++	+++
CNTF	ND	ND	+++
GFAP	+	+++	+++
S100b	+	++	+++
NT3 Neurotrophin	+	+	+++
GLAST	+	++	+++
GLUL	+	++	+++
GLT1	+	++	+++
GAPDH	+	+	+

Thus, it can be seen that the differentiated cells of the present invention show a specific phenotype that is different to both mesenchymal stem cells and astrocytes. For example, the cells of the present invention express of CD90, tyrosine hydroxylase and H-NF differentiating them from astrocytes. In addition, the cells of the present invention express high levels of neurotrophic factors and astrocyte markers, differentiating them from mesenchymal stem cells.

EXAMPLE 2

Intrastriatal transplantation of astrocyte-like differentiated hBMSc improve the motor functions of lesion rats

In order to explore the possible therapeutic potential of the cells of the present invention, HBMSCs differentiated according to the method of the present invention were transplanted in an established rat model of Parkinson disease.

MATERIALS AND METHODS

Animals: Male Sprague–Dawley rats (Harlan, Israel) weighing 220–280 g were used for the 6-OHDA-lesion experiment. All animals were housed in standard conditions; constant temperature (22 ± 1 °C), humidity (relative, 30 %), 12-h light: 12-h dark cycle and free access to food and water. Surgical procedures were performed under the supervision of the Animal Care Committee at the Rabin Medical Center and at Tel Aviv University, Tel Aviv, ISRAEL.

6-OHDA lesions: Rats were anaesthetized with chloral hydrate, 350 mg/kg i.p., and secured in a stereotaxic frame (Stoelting, USA). Animals were unilaterally injected with 6-OHDA hydrobromide (12 µg /6 µl dissolved in ascorbate-saline) using Hamilton 10 µl syringe with a 26-gauge needle, into the left striatum at the two sites (6µl per each site in two depth). The coordinates of the injections were as follows: (1) AP: +0.5, mm from bregma, L: –2.5, mm lateral to middle, V: –6.5 mm ventral to the dural surface, and (2) AP: –0.5 mm, L: –3.7 mm, V: –6.0 mm , based on the stereotaxis atlas (Paxinos & Watson, 1986). At the completion of the injection, the cannula was left in place for another 3 minutes before being withdrawn at 1 mm/min in order to prevent a vacuum.

Measurement of rotational behaviour: Lesioned rats were tested for rotation behavior 14 days following the intracerebral injection of 6-OHDA. Motor asymmetry was monitored in an automated rotation-measuring apparatus for 1 hour following

subcutaneous injection of the DA agonist apomorphine (Sigma, 0.15 mg/kg, dissolved in normal saline). Apomorphine induces contralateral turning behavior to the original lesion side because the striatal DA receptors become hypersensitive as a result of the 6-OHDA lesion. Rats with a rotation rate of five turns per minute were considered to be an established PD model and were used for grafting experiments. This test is widely used as a reliable index of dopamine depletion in the striatum.

Transplantation: Six weeks following the 6-OHDA lesion, the PD model rats were divided into two groups, a control group and an experimental group. Rats in the control group (n=7) were injected with 0.9 % Saline. Rats in the experimental group (n=6) were grafted with GDNF-producing cells. hBMSC astrocyte like cells were harvested prior to transplantation and resuspended at 5×10^5 vital cells/5µl in saline. The cells were stereotactically injected into the left striatum in one site at the two depth (2.5 µl per each) through a 26-gauge needle of a 10 µl Hamilton syringe. The coordinates used for injection were as follows: (1) AP: +1.0 mm, L: +3.0 mm, V: –5.0 mm; and (2) AP: +1.0 mm, L: +3.0 mm, V: –4.1 mm, with the tooth-bar set at the level of the interaural line. After the operation, the animals were subjected to rotation tests every two week as described above. In the case of human MSC transplantation, animals were immunosuppressed by subcutaneous injection of Sandimune (10 mg/kg/day; Novartis) every day.

Rotor-rod: Performance on a rotorod task was tested at 3 months post transplantation. The task consisted of three consecutive trials in which the rat were placed on the rod and challenged (until 16 RPM) to prevent themselves from falling off the accelerating rod. Each trial was continued up to a maximum of 2 minutes. The interval between trials was 30 seconds. The results were obtained from two weekly tests.

Sun flower test: The purpose of this test was to challenge the animals to open sunflowers seeds during a maximum time of 5 minutes. In order to perform the test animal were denied excess food and supplied with only 45 mg/gk/day three days prior to initiation of monitoring. Animals were trained for 3 days and on the fourth and fifth day, measurements were taken. The animals were placed in empty cages and supplied with seeds for 5 minutes. The number of open and eaten seeds was measured. [Gonzalez C., Kolb B. A comparison of different models of stroke on behavior and brain morphology. E. J. Neuroscience 18 (1950-1962),2003].

Immunohistochemistry: At 16 weeks following transplantation both the control and treatment groups were subjected to immunostaining. Rats were deeply anaesthetized with chloral hydrate (350 mg/kg i.p) and perfused transcardially with 65 ml of cold saline, followed by 250 ml of cold 4 % paraformaldehyde in 0.1 mol/l PBS (pH 7.4). Brains were removed, placed in 4 % PFA/PBS for 48 hours, followed by cryoprotection in 20 % sucrose/PBS for 48 hours, and then frozen in isopentane on dry ice. Serial coronal sections of the brain were cut at 20 μm thickness on a microtome cryostat. The sections were washed 3 times in PBS, and subsequently blocked in 5 % normal horse serum (Biological Industries) in PBS for 1 hour. Subsequently, the sections were incubated for 24 hours in the same solution containing primary antibodies against human nuclei (1:30; mouse monoclonal; Chemicon International, Temecula, CA, USA), against GDNF (1:200; rabbit polyclonal, Santa Cruz) and against GFAP (1:100, rabbit DAKO). Sections were then washed three times in PBS and exposed to secondary antibodies: goat anti-mouse IgG (1:500; Jackson Immuno Research Laboratories Inc., West Grove, PA, USA) coupled to rhodamine; and goat anti-rabbit IgG conjugated to Alexa-488 (1:500 Molecular Probes, Eugene, OR, USA) applied for 1 hour at room temperature. Nucleii were stained for 5 minutes with the nuclear dye DAPI 1:200 (Sigma). Finally, sections were rinsed in 0.1 M PBS and coverslipped with 95 % glycerol. Control immunostaining experiments were carried out by either omitting: (i) primary antibody, (ii) secondary antibody, and (iii) both primary and secondary antibodies.

RESULTS

6-hydroxydopamine was injected intra striatally into rat brains to induce moderate damage of the dopaminergic neurons in the striatum. The extent of the lesion was measured three weeks later by injection of apomorphine leading to the ipsilateral rotations. Astrocyte-like cells ($5X10^5$) were grafted into the ipsilateral striatum of rats that were preselected based on their rotational performance. Pharmacological- induced rotational behavior was measured in transplanted rats as well as in saline injected rats at 60, 75 and 105 days following engraftment. During the experiment, both groups were injected daily with cyclosporine in order to prevent graft rejection. As illustrated in Figure 11, apomorphine-induced rotations decreased from an average of 160 rotations per hour at baseline to 60 rotations per hour at 75 days in transplanted rats (n=8), and 50 rotations per hour 105 days following

transplantation (68 % and 75 % reduction $p<0.05$). The control group (n=8) rotated 155 times per hour at baseline and 140 after 105 days (no significant reduction).

95 days following transplantation, a non-pharmacological rotor-rod test was performed in order to provide direct measurement of motor deficits. Animals transplanted with the astrocyte-like cells were able to walk for a longer period on the accelerating rod than the control (Figure 12).

The sun flower eating test was also applied to the animals. At day 100 following transplantation animals were evaluated for their ability to open sun flower seeds in a limited time (see methods). The transplanted animals opened 44 seeds in 5 minutes compared to control that opened 44 in the same time ($p<0.05$) (Figure 13).

At 110 days following transplantation the animals were sacrificed and histology studies were performed in order to identify and characterize the transplanted cells. Anti-human nuclear antigen and anti-GFAP antibodies were used to identify the transplanted differentiated cells (Figures 14A-E). Immunostaining and confocal microscopy studies revealed that up to 25 % of the cells that were positive for human antigen were also positive for GFAP. These results indicate that the astrocyte-like cells are capable of restoring the dopamine deficit in the striatum and are probably of benefit in other neurodegenerative disease.

EXAMPLE 3

Intramuscular transplantation of astrocyte-like differentiated hBMSc improve the motor functions of a transgenic mouse model of ALS

MATERIALS AND METHODS

Animal Model: The colony of TgN(SOD1-G93A)1Gur transgenic mice (Gurney 1994) was obtained from the Jackson Laboratory (USA). The mice were bred in CSJLF1 and at one month of age offspring was genotyped by PCR analysis, using the following PCR primers: hSOD1 upstream primer (SEQ ID NO: 19) 5`CTAGGCCACAGAATTGAAAGATCT3`; hSOD1 downstream primer (SEQ ID NO: 20) 5`GTAGGTGGAAATTCTAGCATCATCC3`; IL2 upstream primer (SEQ ID NO: 21) 5`CATCAGCCCTAATCCATCTGA3`; IL2 downstream primer (SEQ ID NO: 22) 5`CGCGACTAACAATCAAAGTGA3`. The annealing temperature was 53 °C, the PCR products 236 bp (hSOD1), 324bp (IL2). Mice to be used in this study were healthy until 3 months of age and became completely paralyzed between 4 and 5

months of age. Animal experiments were approved and supervised by the Tel-Aviv University.

Cell transplantation and behavior: On day 40, $0.5x10^6$ human astrocyte differentiated cells (prepared as described in Example 1) were transplanted intra gastrocnemius muscle in two legs. The motor performance was evaluated every two week by a rotarod apparatus with accelerated rotations.

Histopathological evaluation: Mice were sacrificed at day 110 and the legs were placed in 4 % paraformaldhyde 0.1 M phosphate buffer, pH 7.4, 4 °C. Paraffin blocks were prepared and immunostained with anti human antigens antibodies.

Statistical analysis: Comparisons were made by ANOVA with post hoc Dunnett test when more than two groups were involved. If data was not normally distributed, a nonparametric test (Mann–Whitney U-test) was used for the comparisons of results. Data was expressed as mean ±s.e.m.

RESULTS

The astrocyte differentiated cells (0.5×10^6) were transplanted on day 40, into the hmSOD mice (n=8) intra muscle (feet). Parallel group was injected with saline. In addition wild type mice (n=8) and mhSOD Tg mice (n=8) were used as positive and negative controls.

The behavioral assessment was performed on rotarod (see M&M) from day 40 and up till death (day 120-140) every two weeks. As seen in Figure 15, the reduction in motor performance (i.e. the ALS symptoms) was significantly delayed in the cell-transplanted mice.

Animal weight was measured and compared between the transplanted group and the controls and statistically analyzed. The control group showed a significantly greater ($p<0.05$) weight reduction than the transplanted group (Figure 16).

A histological study that was performed on day 110 with anti-human nuclear antigen antibodies indicated that the engrafted cells survived (Figure 17A-C).

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

WHAT IS CLAIMED IS:

1. An isolated human cell comprising at least one astrocytic phenotype and at least one mesenchymal stem cell phenotype, wherein said mesenchymal stem cell phenotype is not an astrocytic phenotype.

2. An isolated human cell comprising at least one mesenchymal stem cell phenotype and at least one astrocytic structural phenotype, wherein said mesenchymal stem cell phenotype is not an astrocytic structural phenotype.

3. An isolated human cell comprising at least one mesenchymal stem cell phenotype and at least one astrocytic functional phenotype, wherein said mesenchymal stem cell phenotype is not an astrocytic functional phenotype.

4. An isolated human cell comprising at least one mesenchymal stem cell phenotype and expressing at least one neurotrophic factor, wherein said expression is at least 2 times greater than a basal expression of said neurotrophic factor in a mesenchymal stem cell.

5. An isolated cell population comprising human cells wherein:

(i) at least N % of said human cells comprise at least one astrocytic phenotype;

(ii) at least M % of said human cells comprise at least one mesenchymal stem cell phenotype, said mesenchymal stem cell phenotype is not an astrocytic phenotype; and(iii) at least one of said human cells comprises both said at least one astrocytic phenotype and said at least one mesenchymal stem cell phenotype;

where M and N are each independently selected between 1 and 99.

6. An isolated cell population comprising human cells wherein:

(i) at least N % of said human cells express at least one neurotrophic factor, wherein said expression is at least 2 times greater than a basal expression of said neurotrophic factor in a mesenchymal stem cell;

(ii) at least M % of said human cells comprise at least one mesenchymal stem cell phenotype; and

(iii) at least one of said human cells expresses said at least one neurotrophic factor and said at least one mesenchymal stem cell phenotype;

where M and N are each independently selected between 1 and 99.

7. An isolated cell population comprising human cells wherein:

(i) at least N % of said human cells comprise at least one astrocytic structural phenotype;

(ii) at least M % of said human cells comprise at least one mesenchymal stem cell phenotype, said mesenchymal stem cell phenotype is not an astrocytic structural phenotype; and

(iii) at least one of said human cells comprise both said at least one astrocytic structural phenotype and said at least one mesenchymal stem cell phenotype;

where M and N are each independently selected between 1 and 99.

8. An isolated cell population comprising human cells wherein:

(i) at least N % of said human cells comprise at least one astrocytic functional phenotype;

(ii) at least M % of said human cells comprise at least one mesenchymal stem cell phenotype, said mesenchymal stem cell phenotype is not an astrocytic functional phenotype; and

(iii) at least one of said human cells comporise both said at least one astrocytic functional phenotype and said at least one mesenchymal stem cell phenotype;

where M and N are each independently selected between 1 and 99.

9. The isolated human cell and isolated cell populations of any of claims 1, 2, 3 4, 5, 6, 7 or 8, wherein the cells are non-genetically manipulated.

10. The isolated human cell or cell population of claims 1 or 5, wherein said at least one astrocytic phenotype is a structural phenotype.

11. The isolated human cell or cell population of claims 1 or 5, wherein said at least one astrocytic phenotype is a functional phenotype.

12. The isolated human cell or cell population of claims 2 or 7, wherein said cells further comprise an astrocytic functional phenotype.

13. The isolated human cell or cell population of claim 12, wherein said astrocytic functional phenotype is not said mesenchymal stem cell phenotype.

14. The isolated human cell or cell population of claims 3 or 8, wherein said cells further comprise an astrocytic structural phenotype.

15. The isolated human cell or cell population of claims 4 or 6, wherein said cells further comprise an astrocytic structural phenotype.

16. The isolated human cell or cell population of claims 14 or 15, wherein said astrocytic structural phenotype is not said mesenchymal stem cell phenotype.

17. The isolated human cell or cell population of any of claims 2, 7, 10, 14 or 15, wherein said astrocytic structural phenotype is a cell size, a cell shape, an organelle size and an organelle number.

18. The isolated human cell or cell population of any of claims 2, 7, 10, 14 or 15, wherein said astrocytic structural phenotype is expression of at least one astrocytic marker.

19. The isolated human cell or cell population of claim 18, wherein said astrocytic marker is a surface marker.

20. The isolated human cell or cell population of claim 18, wherein said astrocytic marker is an internal marker.

21. The isolated human cell or cell population of any of claims 3, 8, 11 or 12, wherein said astrocytic functional phenotype is expression of at least one neurotrophic factor at a level at least 2 times greater than a basal production of said neurotrophic factor in a mesenchymal stem cell.

22. The isolated human cell or cell population of any of claims 4, 6 or 21, wherein said at least one neurotrophic factor is selected from the group consisting of glial derived neurotrophic factor (GDNF), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4/5, Neurturin (NTN), Persephin, brain derived neurotrophic factor (BDNF), artemin (ART), ciliary neurotrophic factor (CNTF), insulin growth factor-I (IGF-1) and Neublastin.

23. The isolated human cell or cell population of claim 22, wherein said at least one neuroptrophic factor is GDNF.

24. The isolated human cell or cell population of claim 18, wherein said astrocyte marker is selected from the group consisting of S100 beta, glial fibrillary acidic protein (GFAP), glutamine sythetase, GLT-1 and GLAST.

25. The isolated human cell or cell population of claim 23, wherein a secretion of said GDNF is regulated by IL-1 beta and/or cabergoline.

26. A method of generating astrocyte-like cells, comprising incubating mesenchymal stem cells in a differentiating medium comprising platelet derived growth factor (PDGF) and human neuregulin 1-β1, thereby generating astrocyte-like cells.

27. The method of claim 26, wherein a duration of said incubating is about 48 hours.

28. The method of claim 26, wherein a concentration of said PDGF is about 5 ng/ml.

29. The method of claim 26, wherein a concentration of said human neuregulin 1-β1 is about 50 ng/ml.

30. The method of claim 26, wherein said differentiating medium further comprises L-glutamine, dibutyryl cyclic AMP and isobutylmethylxanthine IBMX.

31. The method of claim 26, further comprising culturing the cells in an additional medium prior to said incubating thereby predisposing said cells to differentiate into astrocyte-like cells.

32. The method of claim 31, wherein said additional medium comprises human epidermal growth factor (hEGF) and human basic fibroblast growth factor (hbFGF).

33. The method of claim 32, wherein a concentration of hEGF is about 20 ng/ml.

34. The method of claim 32, wherein a concentration of hbFGF is about 20 ng/ml.

35. The method of claim 31, wherein said additional medium further comprises L-glutamine, insulin, progesterone, putrescin, selenium and transferrin.

36. The method of claim 31, wherein a duration of said culturing is about 48 hours.

37. The method of claim 26, wherein said mesenchymal stem cells are obtained by:

(a) culturing a population of cells comprising said mesenchymal stem cells in a proliferating medium capable of maintaining and/or expanding said mesenchymal stem cells; and

(b) selecting said mesenchymal stem cells from the cells resulting from step (a).

38. The method of claim 37, wherein step (b) is affected by harvesting surface adhering cells.

39. A method of generating astrocyte-like cells, comprising incubating mesenchymal stem cells in a medium comprising at least one differentiating agent, said at least one differentiating agent being selected from the group consisting of

platelet derived growth factor (PDGF), human neuregulin 1-β1, FGF2, EGF, N2, IBMX and cAMP, thereby generating astrocyte-like cells.

40. A method of treating a CNS disease or disorder comprising administering to an individual in need thereof a therapeutically effective amount of astrocyte-like cells, thereby treating the CNS disease or disorder.

41. Use of astrocyte-like cells for the treatment of a CNS disease or disorder.

42. The method of claim 40, further comprising administering to said individual, stem cells capable of endogenously synthesizing at least one neurotransmitter.

43. The method or use of claims 40 or 41, wherein the CNS disease or disorder is a neurodegenerative disease or disorder.

44. The method or use of claims 40 or 41, wherein the CNS disease or disorder is selected from the group consisting of a motion disorder, a dissociative disorder, a mood disorder, an affective disorder, an addictive disorder and a convulsive disorder.

45. The method or use of claim 43, wherein the neurodegenerative disorder is selected from the group consisting of Parkinson's, multiple sclerosis, epilepsy, amyatrophic lateral sclerosis, stroke, autoimmune encephalomyelitis, diabetic neuropathy, glaucatomus neuropathy, Alzheimer's disease and Huntingdon's disease.

46. The method or use of claims 40 or 41, wherein said cells are autologous cells.

47. The method or use of claims 40 or 41, wherein said cells are non-autologous cells.

48. A pharmaceutical composition comprising as an active agent any of the cells or cell populations of any of claims 1, 2, 3, 4, 5, 6, 7 or 8 and a pharmaceutically acceptable carrier.