It has also been found that it is possible to alter the production of microparticles by stem cells by the addition of components to the culture medium, by culturing the stem cells under hypoxic conditions, or by co-culture with other cell types, thereby providing an improved method of producing stem cell microparticles.

In one aspect, the present invention refers to a method of producing a stem cell microparticle, comprising: i) culturing the stem cells in an environment that allows stem cell differentiation, which is a multi-compartment bioreactor; and ii) collecting the microparticles that are produced by the cells. In another aspect, the present invention refers to a microparticle: i) obtainable by the method as described herein; or (ii) which is an exosome and the exosome expresses one or more of: DCX, GFAP, GALC, TUBB3, GDNF and IDO; and wherein: (a) the exosome expresses one, two, three, four, five, six, seven, eight, nine, ten or more or more of the following miRNAs at a higher level than is expressed in the corresponding stem cells cultured in standard T-175 flasks, as calculated by Fold Regulation: hsa-miR-146b-5p; hsa-let-7c; hsa-miR-99a; hsa-miR-132; hsa-miR-378; hsa-miR-181a; hsa-let-7b; hsa-miR-100; hsa-let-7e; hsa-miR-23b; hsa-miR-185; hsa-let-7i; hsa-let-7a; hsa-let-7d; hsa-let-7g; hsa-miR-222; hsa-let-7f; hsa-miR-218; hsa-miR-24; hsa-miR-9; hsa-miR-126; hsa-miR-134; hsa-miR-128; and hsa-miR-155; (b) the exosome expresses one, two, three, four, five, six, seven, eight, nine, ten or more of the following miRNAs at a lower level than is expressed in the corresponding stem cells cultured in standard T-175 flasks, as calculated by Fold Regulation: hsa-miR-22; hsa-miR-26a; hsa-miR-210; hsa-miR-92a; hsa-miR-93; hsa-miR-424; hsa-miR-195; hsa-miR-127-5p; hsa-miR-21; hsa-miR-103a; hsa-miR-16; hsa-miR-125a-5p; hsa-miR-10a; hsa-miR-10b; hsa-miR-345; hsa-miR-130a; hsa-miR-15b; hsa-miR-20b; hsa-miR-20a; hsa-miR-17; hsa-miR-7; hsa-miR-106b; hsa-miR-101; hsa-miR-302a; hsa-miR-301a; hsa-miR-183; hsa-miR-219-5p; hsa-miR-18a; hsa-miR-15a; hsa-miR-182; hsa-miR-33a; hsa-miR-96; and hsa-miR-18b; and/or (c) the microparticle comprises one, two, three or four of hsa-miR-1246, hsa miR-4492, hsa-miR-4488 and hsa-miR-4532.

In ~~a first aspect~~one example, the present ~~invention refers to~~ disclosure provides a method of producing a stem cell microparticle, comprising: i) culturing the stem cells in an environment that allows stem cell differentiation; and ii) collecting the microparticles that are produced by the cells. In ~~a second aspect~~another example, the present disclosure provides ~~invention refers to~~ a microparticle obtainable by the method ~~of any one of the present invention~~as described herein. In ~~a third aspect~~yet another example, the present disclosure provides ~~invention refers to~~ a microparticle which is an exosome and the exosome expresses one or more of: DCX, GFAP, GALC, TUBB3, GDNF and IDO. In ~~a fourth aspect~~aspect further example, the present disclosure provides ~~invention refers to~~ a composition comprising the microparticle of the present

invention and a stem cell, optionally wherein the stem cell is the stem cell from which the microparticle is derived, for example wherein the stem cell is CTX0E03 having ECACC Accession No. 04091601.

One example provides a neural stem cell microparticle. The microparticle may be an exosome, microvesicle, membrane particle, membrane vesicle, exosome-like vesicle, ectosome-like vesicle, ectosome or exovesicle. Typically, the microparticle is an exosome. The microparticle may be derived from a neural stem cell that has been cultured in an environment that allows stem cell differentiation. The microparticle may be isolated from partially-differentiated neural stem cells. In one example, an environment that allows stem cell differentiation is a multi-compartment bioreactor, typically where the cells are cultured for more than seven days. The microparticle may be derived from a neural stem cell line. In some examples, the neural stem cell line may be the "CTX0E03" cell line, the "STR0C05" cell line, the "HPC0A07" cell line or the neural stem cell line disclosed in Miljan et al Stem Cells Dev. 2009. In some examples, the microparticle is derived from a stem cell line that does not require serum to be maintained in culture. The microparticle may have a size of between 30 nm and 1000 nm, or between 30 and 200 nm, or between 30 and 100 nm, as determined by electron microscopy; and/or a density in sucrose of 1.1-1.2 g/ml. The microparticle may comprise RNA. The RNA may be mRNA, miRNA, and/or any other small RNA. The microparticle may comprise one, two, three or four of hsa-miR-1246, hsa-miR-4492, hsa-miR-4488 and hsa-miR-4532. The microparticle may comprise one or more lipids, typically selected from ceramide, cholesterol, sphingomyelin, phosphatidylserine, phosphatidylinositol, phosphatidylcholine. The microparticle may comprise one or more tetraspanins, typically CD63, CD81, CD9, CD53, CD82 and/or CD37. The microparticle may comprise one or more of TSG101, Alix, CD109, thy-1 and CD133. The microparticle may comprise at least 10 of the proteins present in Table 19 or Table 21. The microparticle may comprise at least one biological activity of a neural stem cell or a neural stem cell-conditioned medium. At least one biological activity may be a tissue regenerative activity. The microparticle as disclosed herein is typically isolated or purified.

A further example of the invention provides a neural stem cell microparticle for use in therapy. The therapy may be regenerative therapy requiring tissue replacement, regeneration or repair, for example where the therapy requires angiogenesis, neurogenesis and/or neuroprotection. The therapy may be for a neurological disease, disorder or deficit. The therapy may improve functional and/or cognitive recovery. The therapy may be of stroke, peripheral arterial disease,

CLAIMS

1. A method of producing a stem cell microparticle, comprising:
    i. culturing the stem cells in an environment that allows stem cell differentiation, which is a multi-compartment bioreactor; and
    ii. collecting the microparticles that are produced by the cells.
2. The method of claim 1, wherein the stem cell is a microparticle-producing stem cell.
3. The method of claim 1, wherein:
    (a) the bioreactor contains at least two compartments separated by one or more membranes or barriers that separate the compartment containing the cells from one or more compartments containing gas and/or culture medium; and/or(b) the culture is for more than seven days.
4. The method of any one of the preceding claims, comprising isolating a microparticle from a stem cell-conditioned medium, optionally wherein the stem cell-conditioned medium comprises one or more additive components or agents which stimulate the release of microparticles by the stem cells into the medium, optionally wherein the one or more components are selected from transforming growth factor-beta (TGF-β), interferon-gamma (INF-γ) and/or tumour necrosis factor-alpha (TNF-α).
5. The method of claim 4, wherein the stem cells were:
    (a) cultured under hypoxic conditions; and/or
    (b) co-cultured with a different cell type, optionally wherein the different cell type is an endothelial cell.
6. The method of any one of the preceding claims, wherein the stem cell is a neural stem cell, optionally wherein the stem cell is a neural stem cell line, optionally which is conditionally-immortalised, for example the neural stem cell line is CTX0E03 having ECACC Accession No. 04091601, STR0C05 having ECACC Accession No.04110301, or HPC0A07 having ECACC Accession No.04092302.
7. The method of claim 4, wherein:
    (a) the neural stem cell line is grown in serum-free medium;
    (b) the neural stem cell expresses one or more of the markers: Nestin, Sox2, GFAP, βIII tubulin, DCX, GALC, TUBB3, GDNF and IDO; and/or
    (c) the microparticle is an exosome and the exosome expresses one or more of: DCX, GFAP, GALC, TUBB3, GDNF and IDO.
8. The method of any one of the preceding claims, wherein:
    (a) at least two, three, four, five, six or seven miRNAs are up or down regulated in the microparticle compared to in the corresponding stem cells cultured in standard T-175 flasks, as calculated by Fold Regulation;

(b) the microparticle is an exosome and the exosome expresses one, two, three, four, five, six, seven, eight, nine, ten or more or more of the following miRNAs at a higher level than is expressed in the corresponding stem cells cultured in standard T-175 flasks, as calculated by Fold Regulation: hsa-miR-146b-5p; hsa-let-7c; hsa-miR-99a; hsa-miR-132; hsa-miR-378; hsa-miR-181a; hsa-let-7b; hsa-miR-100; hsa-let-7e; hsa-miR-23b; hsa-miR-185; hsa-let-7i; hsa-let-7a; hsa-let-7d; hsa-let-7g; hsa-miR-222; hsa-let-7f; hsa-miR-218; hsa-miR-24; hsa-miR-9; hsa-miR-126; hsa-miR-134; hsa-miR-128; and hsa-miR-155;

(c) the microparticle is an exosome and the exosome expresses one, two, three, four, five, six, seven, eight, nine, ten or more of the following miRNAs at a lower level than is expressed in the corresponding stem cells cultured in standard T-175 flasks, as calculated by Fold Regulation: hsa-miR-22; hsa-miR-26a; hsa-miR-210; hsa-miR-92a; hsa-miR-93; hsa-miR-424; hsa-miR-195; hsa-miR-127-5p; hsa-miR-21; hsa-miR-103a; hsa-miR-16; hsa-miR-125a-5p; hsa-miR-10a; hsa-miR-10b; hsa-miR-345; hsa-miR-130a; hsa-miR-15b; hsa-miR-20b; hsa-miR-20a; hsa-miR-17; hsa-miR-7; hsa-miR-106b; hsa-miR-101; hsa-miR-302a; hsa-miR-301a; hsa-miR-183; hsa-miR-219-5p; hsa-miR-18a; hsa-miR-15a; hsa-miR-182; hsa-miR-33a; hsa-miR-96; and hsa-miR-18b; and/or

(d) the microparticle comprises one, two, three or four of hsa-miR-1246, hsa-miR-4492, hsa-miR-4488 and hsa-miR-4532.

9. The method of any one of the preceding claims, further comprising loading the isolated or purified microparticle with one or more exogenous nucleic acids, lipids, proteins, drugs or prodrugs, optionally wherein the exogenous nucleic acid is siRNA capable of silencing one or more pathological genes.

10. A microparticle:

(i) obtainable by the method of any one of claims 1-9; or

(ii) which is an exosome and the exosome expresses one or more of: DCX, GFAP, GALC, TUBB3, GDNF and IDO; and

wherein:

(a) the exosome expresses one, two, three, four, five, six, seven, eight, nine, ten or more or more of the following miRNAs at a higher level than is expressed in the corresponding stem cells cultured in standard T-175 flasks, as calculated by Fold Regulation: hsa-miR-146b-5p; hsa-let-7c; hsa-miR-99a; hsa-miR-132; hsa-miR-378; hsa-miR-181a; hsa-let-7b; hsa-miR-100; hsa-let-7e; hsa-miR-23b; hsa-miR-185; hsa-let-7i; hsa-let-7a; hsa-let-7d; hsa-let-7g; hsa-miR-222; hsa-let-7f; hsa-miR-218; hsa-miR-24; hsa-miR-9; hsa-miR-126; hsa-miR-134; hsa-miR-128; and hsa-miR-155;

(b) the exosome expresses one, two, three, four, five, six, seven, eight, nine, ten or more of the following miRNAs at a lower level than is expressed in the corresponding stem cells cultured in standard T-175 flasks, as calculated by Fold Regulation: hsa-miR-22; hsa-miR-26a; hsa-miR-210; hsa-miR-92a; hsa-miR-93; hsa-miR-424; hsa-miR-195; hsa-miR-127-5p; hsa-miR-21; hsa-miR-103a; hsa-miR-16; hsa-miR-125a-5p; hsa-miR-10a; hsa-miR-10b; hsa-miR-345; hsa-miR-130a; hsa-miR-15b; hsa-miR-20b; hsa-miR-20a; hsa-miR-17; hsa-miR-7; hsa-miR-106b; hsa-miR-101; hsa-miR-302a; hsa-miR-301a; hsa-miR-183; hsa-miR-219-5p; hsa-miR-18a; hsa-miR-15a; hsa-miR-182; hsa-miR-33a; hsa-miR-96; and hsa-miR-18b; and/or

(c) the microparticle comprises one, two, three or four of hsa-miR-1246, hsa-miR-4492, hsa-miR-4488 and hsa-miR-4532.

11. The microparticle of claim 10, for use in therapy.

12. The microparticle for use according to claim 11, wherein the therapy is for a:

i. Neurological disorder, disease or deficit, such as Parkinson's, Alzheimer's, Stroke, or ALS;

ii. Lysosomal storage disorder;

iii. Cardiovascular disorder, such as Myocardial Infarction, congestive heart failure, Peripheral Arterial Disease, diabetic ulcers, wound healing;

iv. Diseases of the lung, including Idiopathic Pulmonary Fibrosis, Respiratory Distress Syndrome, Chronic Obstructive Pulmonary Disease, Idiopathic Pulmonary Hypertension, Cystic Fibrosis and Asthma;

v. Metabolic or inflammatory disorder, such as Diabetes (I or II), rheumatoid arthritis, osteoarthritis, lupus, Crohn's disease, Irritable Bowel Disease, or Graft versus Host Disease;

vi. Psychiatric disorder, such as: Depression, Bipolar, Schizophrenia or an Autistic syndrome disorder such as Autism, Asperger's syndrome or Rett Syndrome;

vii. Blindness-causing disease of the retina, such as Age-related macular degeneration, Stargardt disease, diabetic retinopathy, or retinitis pigmentosa; or

viii. Demyelinating disease, such as multiple sclerosis, cerebral palsy, central pontine myelinolysis, tabes dorsalis, transverse myelitis, Devic's disease, progressive multifocal leukoencephalopathy, optic neuritis, leukodystrophies, Guillain-Barre syndrome, Anti-MAG peripheral neuropathy and Charcot-Marie-Tooth disease.

13. A composition comprising the microparticle of claim 10 and a stem cell, optionally wherein the stem cell is the stem cell from which the microparticle is derived, for example wherein the stem cell is CTX0E03 having ECACC Accession No. 04091601, for use in therapy.

14. The composition for use according to claim 13, wherein the stem cell and the microparticle are administered:
    i. together in a single pharmaceutical composition;
    ii. contemporaneously or simultaneously but separately; or
    iii. separately and sequentially, for example wherein the duration between the administration of the cell and microparticle is one hour, one day, one week, two weeks or more.
15. The composition for use according to any one of claims 13-14, wherein the therapy induces tolerance, typically immunotolerance, in a host that is to receive the stem cells from which the microparticle is derived; or wherein tolerance to the stem cells is increased by administering stem cells together with the microparticles.

CLAIMS

1. A method of producing a stem cell microparticle, comprising:
    i. culturing the stem cells in an environment that allows stem cell differentiation; and
    ii. collecting the microparticles that are produced by the cells.
2. The method of claim 1, wherein an environment that allows stem cell differentiation is culture in a multi-compartment bioreactor, for example wherein the bioreactor contains at least two compartments separated by one or more membranes or barriers that separate the compartment containing the cells from one or more compartments containing gas and/or culture medium.
3. The method of claim 2, wherein the culture is for more than seven days.
4. The method of any one of the preceding claims, comprising isolating a microparticle from a stem cell-conditioned medium.
5. The method of claim 4, wherein the stem cell-conditioned medium comprises one or more additive components or agents which stimulate the release of microparticles by the stem cells into the medium.
6. The method of claim 5, wherein the one or more components are selected from transforming growth factor-beta (TGF-β), interferon-gamma (INF-γ) and/or tumour necrosis factor-alpha (TNF-α).
7. The method of any one of claims 4-6, wherein the stem cells were cultured under hypoxic conditions.
8. The method of any one of claims 4-7, wherein the stem cells were co-cultured with a different cell type.
9. The method of claim 8, wherein the different cell type is an endothelial cell.
10. The method of any one of the preceding claims, wherein the stem cell is a neural stem cell.
11. The method of claim 10, wherein the stem cell is a neural stem cell line.
12. The method of claims 11, wherein the neural stem cell line is conditionally-immortalised.
13. The method of claims 11 or 12, wherein the neural stem cell line is CTX0E03 having ECACC Accession No. 04091601, STR0C05 having ECACC Accession No.04110301, or HPC0A07 having ECACC Accession No.04092302.
14. The method of any one of claims 11-13, wherein the neural stem cell line is grown in serum-free medium.
15. The method of any one of claims 10-14, wherein the neural stem cell expresses one or more of the markers: Nestin, Sox2, GFAP, βIII tubulin, DCX, GALC, TUBB3, GDNF and IDO.
16. The method of any one of claims 10-15, wherein the microparticle is an exosome and the exosome expresses one or more of: DCX, GFAP, GALC, TUBB3, GDNF and IDO.

17. The method of any one of the preceding claims, wherein at least two, three, four, five, six or seven miRNAs are up or down regulated in the microparticle compared to in the corresponding stem cells cultured in standard T-175 flasks, as calculated by Fold Regulation.

18. The method of any one of the preceding claims, wherein the microparticle is an exosome and the exosome expresses one, two, three, four, five, six, seven, eight, nine, ten or more or more of the following miRNAs at a higher level than is expressed in the corresponding stem cells cultured in standard T-175 flasks, as calculated by Fold Regulation: hsa-miR-146b-5p; hsa-let-7c; hsa-miR-99a; hsa-miR-132; hsa-miR-378; hsa-miR-181a; hsa-let-7b; hsa-miR-100; hsa-let-7e; hsa-miR-23b; hsa-miR-185; hsa-let-7i; hsa-let-7a; hsa-let-7d; hsa-let-7g; hsa-miR-222; hsa-let-7f; hsa-miR-218; hsa-miR-24; hsa-miR-9; hsa-miR-126; hsa-miR-134; hsa-miR-128; and hsa-miR-155.

19. The method of any one of the preceding claims, wherein the microparticle is an exosome and the exosome expresses one, two, three, four, five, six, seven, eight, nine, ten or more of the following miRNAs at a lower level than is expressed in the corresponding stem cells cultured in standard T-175 flasks, as calculated by Fold Regulation: hsa-miR-22; hsa-miR-26a; hsa-miR-210; hsa-miR-92a; hsa-miR-93; hsa-miR-424; hsa-miR-195; hsa-miR-127-5p; hsa-miR-21; hsa-miR-103a; hsa-miR-16; hsa-miR-125a-5p; hsa-miR-10a; hsa-miR-10b; hsa-miR-345; hsa-miR-130a; hsa-miR-15b; hsa-miR-20b; hsa-miR-20a; hsa-miR-17; hsa-miR-7; hsa-miR-106b; hsa-miR-101; hsa-miR-302a; hsa-miR-301a; hsa-miR-183; hsa-miR-219-5p; hsa-miR-18a; hsa-miR-15a; hsa-miR-182; hsa-miR-33a; hsa-miR-96; and hsa-miR-18b.

20. The method of any of the preceding claims, wherein the microparticle comprises one, two, three or four of hsa-miR-1246, hsa-miR-4492, hsa-miR-4488 and hsa-miR-4532.

21. The method of any one of the preceding claims, further comprising loading the isolated or purified microparticle with one or more exogenous nucleic acids, lipids, proteins, drugs or prodrugs.

22. The method of claim 21, wherein the exogenous nucleic acid is siRNA capable of silencing one or more pathological genes.

23. A microparticle obtainable by the method of any one of claims 1-22.

24. A microparticle which is an exosome and the exosome expresses one or more of: DCX, GFAP, GALC, TUBB3, GDNF and IDO.

25. The microparticle of any one of claims 23-24, wherein the exosome expresses one, two, three, four, five, six, seven, eight, nine, ten or more or more of the following miRNAs at a higher level than is expressed in the corresponding stem cells cultured in standard T-175 flasks, as calculated by Fold Regulation: hsa-miR-146b-5p; hsa-let-7c; hsa-miR-99a; hsa-miR-132; hsa-miR-378; hsa-miR-181a; hsa-let-7b; hsa-miR-100; hsa-let-7e; hsa-miR-23b; hsa-miR-185; hsa-let-7i; hsa-let-7a; hsa-let-7d; hsa-let-7g; hsa-miR-222; hsa-

let-7f; hsa-miR-218; hsa-miR-24; hsa-miR-9; hsa-miR-126; hsa-miR-134; hsa-miR-128; and hsa-miR-155.

26. The microparticle of any one of claims 23-25, wherein the exosome expresses one, two, three, four, five, six, seven, eight, nine, ten or more of the following miRNAs at a lower level than is expressed in the corresponding stem cells cultured in standard T-175 flasks, as calculated by Fold Regulation: hsa-miR-22; hsa-miR-26a; hsa-miR-210; hsa-miR-92a; hsa-miR-93; hsa-miR-424; hsa-miR-195; hsa-miR-127-5p; hsa-miR-21; hsa-miR-103a; hsa-miR-16; hsa-miR-125a-5p; hsa-miR-10a; hsa-miR-10b; hsa-miR-345; hsa-miR-130a; hsa-miR-15b; hsa-miR-20b; hsa-miR-20a; hsa-miR-17; hsa-miR-7; hsa-miR-106b; hsa-miR-101; hsa-miR-302a; hsa-miR-301a; hsa-miR-183; hsa-miR-219-5p; hsa-miR-18a; hsa-miR-15a; hsa-miR-182; hsa-miR-33a; hsa-miR-96; and hsa-miR-18b.
27. The microparticle of any one of claims 23-26, wherein the microparticle comprises one, two, three or four of hsa-miR-1246, hsa-miR-4492, hsa-miR-4488 and hsa-miR-4532.
28. The microparticle of any one of claims 23-27, for use in therapy.
29. The microparticle or claim 28, wherein the therapy is for a:
    i. Neurological disorder, disease or deficit, such as Parkinson's, Alzheimer's, Stroke, or ALS;
    ii. Lysosomal storage disorder;
    iii. Cardiovascular disorder, such as Myocardial Infarction, congestive heart failure, Peripheral Arterial Disease, diabetic ulcers, wound healing;
    iv. Diseases of the lung, including Idiopathic Pulmonary Fibrosis, Respiratory Distress Syndrome, Chronic Obstructive Pulmonary Disease, Idiopathic Pulmonary Hypertension, Cystic Fibrosis and Asthma;
    v. Metabolic or inflammatory disorder, such as Diabetes (I or II), rheumatoid arthritis, osteoarthritis, lupus, Crohn's disease, Irritable Bowel Disease, or Graft versus Host Disease;
    vi. Psychiatric disorder, such as: Depression, Bipolar, Schizophrenia or an Autistic syndrome disorder such as Autism, Asperger's syndrome or Rett Syndrome;
    vii. Blindness-causing disease of the retina, such as Age-related macular degeneration, Stargardt disease, diabetic retinopathy, or retinitis pigmentosa; or
    viii. Demyelinating disease, such as multiple sclerosis, cerebral palsy, central pontine myelinolysis, tabes dorsalis, transverse myelitis, Devic's disease, progressive multifocal leukoencephalopathy, optic neuritis, leukodystrophies, Guillain-Barre syndrome, Anti-MAG peripheral neuropathy and Charcot-Marie-Tooth disease.
30. A composition comprising the microparticle of any one of claims 23-27 and a stem cell, optionally wherein the stem cell is the stem cell from which the microparticle is derived, for example wherein the stem cell is CTX0E03 having ECACC Accession No. 04091601.
31. The composition of claim 30, for use in therapy.

32. The composition of claim 31, wherein the stem cell and the microparticle are to be administered:
    i. together in a single pharmaceutical composition;
    ii. contemporaneously or simultaneously but separately; or
    iii. separately and sequentially, for example wherein the duration between the administration of the cell and microparticle is one hour, one day, one week, two weeks or more.
33. The composition of any one of claims 31-32, wherein the therapy induces tolerance, typically immunotolerance, in a host that is to receive the stem cells from which the microparticle is derived; or wherein tolerance to the stem cells is increased by administration of stem cells together with the microparticles.