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 from partially-differentiated stem cells, 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 partially-differentiated stem 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 one example, the present 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 another example, the present disclosure provides a microparticle obtainable by the method as described herein. In yet another example, the present disclosure provides a microparticle which is an exosome and the exosome expresses one or more of: DCX, GFAP, GALC, TUBB3, GDNF and IDO. In aspect further example, the present disclosure provides a composition comprising the microparticle of the present

CLAIMS

1. A method of producing a stem cell microparticle from partially-differentiated stem cells, comprising:
    i. culturing 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 partially-differentiated stem 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 is a stem cell line optionally as defined in claim 6 and 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 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.

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, 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.