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.

One aspect of the invention provides an isolated neural stem cell microparticle, which comprises one, two, three or four of hsa-miR-1246, hsa-miR-4492, hsa-miR-4488 and hsa-miR-4532. 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 is typically isolated or purified.

In one example, disclosed herein is an isolated 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,

In one aspect, the invention refers to a method of producing an isolated neural stem cell microparticle as defined herein, comprising isolating thea microparticle from a neural stem cell-conditioned medium. In another aspect, the invention refers to a method of producing a stem cell microparticle, comprising isolating a microparticle from a stem cell-conditioned medium wherein: (i) the stem cell-conditioned medium comprises one or more components which induce the release of microparticles by the stem cells into the medium, wherein the one or more components are selected from: transforming growth factor-beta (TGF-β), interferon-gamma (INF-γ) and tumour necrosis factor-alpha (TNF-α); (ii) ~~the stem cells were cultured under hypoxic conditions; (iii)~~ the stem cells were co-cultured with a different cell type, optionally wherein the different cell type is an endothelial cell; and/or (iii) ~~(iv)~~ the stem cells were cultured in a multi-compartment bioreactor; optionally wherein the stem cell is a neural stem cell, optionally as defined herein~~and/or (v) the stem cells were partially-differentiated~~. In one example, disclosed herein is a method of producing a stem cell microparticle, typically a neural stem cell microparticle. The method may comprise culturing the stem cells in an environment that allows stem cell differentiation and collecting the microparticles that are produced by the cells. The microparticles may be isolated from partially-differentiated neural stem cells. The stem cells may be cultured under conditions that allow the efficient removal of metabolic waste. In one example, an environment that allows stem cell differentiation is culture in a multi-compartment bioreactor, typically for a prolonged period of time (for example more than seven days). The method may comprise isolating a microparticle from a stem cell-conditioned medium. The stem cell-conditioned medium may comprise one or more additive components or agents which stimulate the release of microparticles by the stem cells into the medium. The one or more components may be selected from transforming growth factor-beta (TGF-β), interferon-gamma (IFN-γ) and/or tumour necrosis factor-alpha (TNF-α). The microparticles may be isolated from stem cell-conditioned medium wherein the stem cells were cultured under hypoxic conditions. The microparticles may be isolated from stem cell-conditioned medium produced by stem cells co-cultured with a different cell type, typically endothelial cells, in order to create the NSC niche environment.

One aspect of the invention provides a microparticle obtainable by a method according to the previous aspect of the invention.

In another aspect, the present invention refers to a composition comprising an isolated microparticle as described herein ~~according to any of claims 1-13 or 26~~ and a pharmaceutically acceptable excipient, carrier or diluent. In one example, there is disclosed herein a composition comprising a neural stem cell microparticle and a pharmaceutically acceptable excipient, carrier or diluent.

Yet another aspect of the invention provides a method of screening for an agent that alters the production of a microparticle by a stem cell, comprising contacting a stem cell with a

candidate agent and observing whether the rate of production of microparticles by the contacted stem cell increases or decreases compared to a control.

In another aspect, the invention refers to a kit for use in a method for producing the microparticle as defined herein comprising: (a) a medium; (b) a neural stem cell, wherein the neural stem cell is CTX0E03 having ECACC Accession No. 04091601, STR0C05 having ECACC Accession No.04110301 or HPC0A07 having ECACC Accession No.04092302; (c) optionally the one or more components as defined herein; (d) optionally the microparticle as defined herein suitable for use as a control; (e) optionally a detection agent suitable for specific detection of the produced microparticles; and (f) instructions for producing the microparticle as defined herein using the kit.

In one example, there is disclosed a kit for use in a method for producing a stem cell microparticle, comprising: (a) a medium suitable for culturing stem cells; (b) a stem cell; (c) optionally the one or more components of the fourth aspect of the invention; (d) optionally a stem cell microparticle suitable for use as a control; (e) optionally a detection agent suitable for specific detection of the produced microparticles; and (f) instructions for producing the stem cell microparticle using the kit.

One aspect of the invention provides a composition comprising two, three or all four of hsa-miR-1246, hsa-miR-4492, hsa-miR-4488 and hsa-miR-4532. This composition is optionally a

CLAIMS

1. An isolated neural stem cell microparticle, which comprises one, two, three or four of hsa-miR-1246, hsa-miR-4492, hsa-miR-4488 and hsa-miR-4532.

2. The isolated neural stem cell microparticle of claim 1, wherein the microparticle is an exosome, microvesicle, membrane particle, membrane vesicle, exosome-like vesicle, ectosome-like vesicle, ectosome or exovesicle.

3. The isolated neural stem cell microparticle of claim 1 or 2, wherein the microparticle is derived from a neural stem cell line, which is optionally conditionally-immortalised and/or grown in serum free medium, and optionally 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.

4. The isolated neural stem cell microparticle of any preceding claim, wherein the microparticle has:

   (a) 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; or
   (b) a density in sucrose of 1.1-1.2 g/ml.

5. The isolated neural stem cell microparticle of any preceding claim, comprising mRNA.

6. The isolated neural stem cell microparticle of any preceding claim, comprising one or more of:

   (a) a lipid selected from ceramide, cholesterol, sphingomyelin, phosphatidylserine, phosphatidylinositol, and/or phosphatidylcholine;
   (b) miRNA selected from hsa-let-7g, hsa-miR-101, hsa-miR-10a, hsa-miR-10b, hsa-miR-126, hsa-miR-128, hsa-miR-129-5p, hsa-miR-130a, hsa-miR-134, hsa-miR-137, hsa-miR-155, hsa-miR-15a, hsa-miR-15b, hsa-miR-16, hsa-miR-17, hsa-miR-182, hsa-miR-183, hsa-miR-185, hsa-miR-18b, hsa-miR-192, hsa-miR-194, hsa-miR-195, hsa-miR-20a, hsa-miR-20b, hsa-miR-210, hsa-miR-218, hsa-miR-301a, hsa-miR-302a, hsa-miR-302c, hsa-miR-345, hsa-miR-375, hsa-miR-378, hsa-miR-7, hsa-miR-9, hsa-miR-93, hsa-miR-96, and hsa-miR-99a;
   (c) a tetraspanin, optionally selected from CD63, CD81,CD9, CD53, CD82 and/or CD37;
   (d) TSG101, Alix, CD109 and/or thy-1; and/or
   (e) CD133.

7. The isolated neural stem cell microparticle of any preceding claim, comprising:
   (a) at least 10 of the proteins present in Table 19 or Table 21; and/or
   (b) at least one biological activity of a neural stem cell or a neural stem cell-conditioned medium, optionally regenerative activity.
8. The isolated neural stem cell microparticle of any preceding claim, for use in therapy.
9. The isolated neural stem cell microparticle of claim 8, wherein the therapy:
   (a) is regenerative therapy;
   (b) 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; and/or
      (viii) Demyelinating disease, such as multiple sclerosis, 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;
   (c) improves functional and/or cognitive recovery; and/or
   (d) is of stroke, peripheral arterial disease or blindness-causing diseases of the retina.
10. The isolated neural stem cell microparticle for use according to claim 8 or claim 9, wherein:

(i) the microparticle is an exosome and therapy is of a disease or condition requiring tissue replacement, regeneration or repair; or

(ii) the microparticle is a microvesicle and the therapy is of a disease requiring angiogenesis or a neurological disease, disorder or deficit.

11. A method of producing an isolated neural stem cell microparticle as defined in any of claims 1-7, comprising isolating the microparticle from a neural stem cell-conditioned medium.

12. A method of producing a stem cell microparticle, comprising isolating a microparticle from a stem cell-conditioned medium wherein:

(i) the stem cell-conditioned medium comprises one or more components which induce the release of microparticles by the stem cells into the medium, wherein the one or more components are selected from: transforming growth factor-beta (TGF-β), interferon-gamma (INF-γ) and tumour necrosis factor-alpha (TNF-α);

(ii) the stem cells were co-cultured with a different cell type, optionally wherein the different cell type is an endothelial cell; and/or

(iii) the stem cells were cultured in a multi-compartment bioreactor;

optionally wherein the stem cell is a neural stem cell, optionally as defined in claim 3.

13. A composition comprising an isolated microparticle according to any of claims 1-7 and a pharmaceutically acceptable excipient, carrier or diluent.

14. A kit for use in a method for producing the microparticle of any of claims 1-7 comprising: (a) a medium; (b) a neural stem cell, wherein the neural stem cell is CTX0E03 having ECACC Accession No. 04091601, STR0C05 having ECACC Accession No.04110301 or HPC0A07 having ECACC Accession No.04092302; (c) optionally the one or more components of claim 12(i); (d) optionally the microparticle of any of claims 1-7 suitable for use as a control; (e) optionally a detection agent suitable for specific detection of the produced microparticles; and (f) instructions for producing the microparticle of any of claims 1-7 using the kit.

15. A method of screening for an agent that alters the rate of production of a microparticle by a stem cell, comprising contacting a stem cell with a candidate agent and observing whether the rate production of microparticles by the contacted stem cell increases or decreases compared to a control.

CLAIMS

1. A neural stem cell microparticle.
2. The neural stem cell microparticle of claim 1, wherein the microparticle is an exosome, microvesicle, membrane particle, membrane vesicle, exosome-like vesicle, ectosome-like vesicle, ectosome or exovesicle.
3. The neural stem cell microparticle of claims 1 or 2, wherein the microparticle is derived from a neural stem cell line.
4. The neural stem cell microparticle of claim 3, wherein the neural stem cell line is conditionally-immortalised and/or grown in serum free medium.
5. The neural stem cell microparticle of claim 4, wherein the neural stem cell line is CTX0E03 having ECACC Accession No. 04091601, STR0C05 having ECACC Accession No.04110301 and HPC0A07 having ECACC Accession No.04092302.
6. The neural stem cell microparticle of any preceding claim, wherein the microparticle has:
   (a) 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; or
   (b) a density in sucrose of 1.1-1.2 g/ml.
7. The neural stem cell microparticle of any preceding claim, comprising RNA.
8. The neural stem cell microparticle of claim 7, wherein the RNA is mRNA and/or miRNA.
9. The neural stem cell microparticle of claim 8, wherein the microparticle comprises one, two, three or four of hsa-miR-1246, hsa-miR-4492, hsa-miR-4488 and hsa-miR-4532.
10. The neural stem cell microparticle of any preceding claim, comprising one or more of:
    (a) a lipid selected from ceramide, cholesterol, sphingomyelin, phosphatidylserine, phosphatidylinositol, and/or phosphatidylcholine;
    (b) miRNA, optionally selected from hsa-let-7g, hsa-miR-101, hsa-miR-10a, hsa-miR-10b, hsa-miR-126, hsa-miR-128, hsa-miR-129-5p, hsa-miR-130a, hsa-miR-134, hsa-miR-137, hsa-miR-155, hsa-miR-15a, hsa-miR-15b, hsa-miR-16, hsa-miR-17, hsa-miR-182, hsa-miR-183, hsa-miR-185, hsa-miR-18b, hsa-miR-192, hsa-miR-194, hsa-miR-195, hsa-miR-20a, hsa-miR-20b, hsa-miR-210, hsa-miR-218, hsa-miR-301a, hsa-miR-302a, hsa-miR-302c, hsa-miR-345, hsa-miR-375, hsa-miR-378, hsa-miR-7, hsa-miR-9, hsa-miR-93, hsa-miR-96, and hsa-miR-99a;
    (c) a tetraspanin, optionally selected from CD63, CD81,CD9, CD53, CD82 and/or CD37;
    (d) TSG101, Alix, CD109 and/or thy-1; and/or
    (e) CD133.
11. The neural stem cell microparticle of any preceding claim, comprising at least 10 of the proteins present in Table 19 or Table 21.
12. The neural stem cell microparticle of any preceding claim, comprising at least one biological activity of a neural stem cell or a neural stem cell-conditioned medium.

13. The neural stem cell microparticle of claim 12, wherein the at least one biological activity is regenerative activity.

14. The neural stem cell microparticle of any preceding claim, for use in therapy.

15. The neural stem cell microparticle of claim 14, wherein the therapy is regenerative therapy.

16. The neural stem cell microparticle of claims 14 or 15, 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; and

(viii) Demyelinating disease, such as multiple sclerosis, 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.

17. The neural stem cell microparticle of claims 14-16, wherein the therapy improves functional and/or cognitive recovery.

18. The neural stem cell microparticle of claims 14-17, wherein the therapy is of stroke, peripheral arterial disease or blindness-causing diseases of the retina.

19. The neural stem cell microparticle of claims 14 to 17, wherein:

(i) the microparticle is an exosome and therapy is of a disease or condition requiring tissue replacement, regeneration or repair; or

(ii) the microparticle is a microvesicle and the therapy is of a disease requiring angiogenesis or a neurological disease, disorder or deficit.

20. Use of a neural stem cell microparticle according to any preceding claim, in the manufacture of a medicament for the treatment of a disease.

21. A method of producing a neural stem cell microparticle as defined in claims 1-13, comprising isolating a microparticle from a neural stem cell-conditioned medium.

22. A method of producing a stem cell microparticle, comprising isolating a microparticle from a stem cell-conditioned medium wherein:
    (i) the stem cell-conditioned medium comprises one or more components which induce the release of microparticles by the stem cells into the medium;
    (ii) the stem cells were cultured under hypoxic conditions;
    (iii) the stem cells were co-cultured with a different cell type;
    (iv) the stem cells were cultured in a multi-compartment bioreactor; and/or
    (v) the stem cells were partially-differentiated.
23. A method according to claim 22, wherein the stem cell is a neural stem cell, optionally as defined in any of claims 3 to 5.
24. A method according to claim 22(i) or claim 23 when dependent upon claim 22(i), wherein the one or more components are selected from: transforming growth factor-beta (TGF-β), interferon-gamma (INF-γ) and tumour necrosis factor-alpha (TNF-α).
25. A method according to claim 22(iii), claim 23 or 24 when dependent upon claim 22(iii), wherein the different cell type is an endothelial cell.
26. A microparticle obtainable by the method of any of claims 22-25.
27. A composition comprising a microparticle according to any of claims 1-13 or 26 and a pharmaceutically acceptable excipient, carrier or diluent.
28. A kit for use in a method for producing the microparticle of any of claims 1-13 or 26 comprising: (a) a medium; (b) a neural stem cell; (c) optionally the one or more components of claims 22 to 24; (d) optionally the microparticle of any of claims 1-13 or 26 suitable for use as a control; (e) optionally a detection agent suitable for specific detection of the produced microparticles; and (f) instructions for producing the microparticle of any of claims 1-13 or 26 using the kit.
29. A method of screening for an agent that alters the rate of production of a microparticle by a stem cell, comprising contacting a stem cell with a candidate agent and observing whether the rate production of microparticles by the contacted stem cell increases or decreases compared to a control.
30. A composition comprising two, three or all four of hsa-miR-1246, hsa-miR-4492, hsa-miR-4488 and hsa-miR-4532.
31. A composition according to claim 30, which is a pharmaceutical composition comprising a pharmaceutically acceptable carrier, diluent, vehicle and/or excipient.
32. A composition according to claim 30 or 31, for use in therapy.
33. A composition for use in therapy according to claim 32, wherein the therapy is as defined in claim 16.