stem cells. The Examples show the successful harvest of microparticles from conditionally immortalised neural stem cells and CD34+ cells. Conditional immortalisation provides a constant supply of clonal cells that produce microparticles such as exosomes. The conditionally immortalised cells are useful as "producer cells" for microparticles such as exosomes, which are typically harvested or isolated from the conditionally-immortalised cells.

A first aspect of the invention provides the use of a conditionally-immortalised cell to produce microparticles. The conditionally-immortalised cell is typically:

a mesenchymal stem cell, optionally selected from a bone marrow derived stem cell, an endometrial regenerative cell, a mesenchymal progenitor cell or a multipotent adult progenitor cell;

a neural stem cell~~, optionally~~ selected from a neurosphere initiating stem cell, or an oligodendrocyte precursor cell;

a haematopoietic stem cell, optionally a CD34+ cell and/or isolated from umbilical cord blood, or optionally a CD34+/CXCR4+ cell; a non-haematopoietic umbilical cord blood stem cell;

a very small embryonic like stem cell (VSEL);

an induced pluripotent stem (iPS) cell;

a fibroblast; or

a dendritic cell.

The microparticle that is produced may be an exosome, microvesicle, membrane particle, membrane vesicle, exosome-like vesicle, ectosome-like vesicle, ectosome or exovesicle. Typically, the microparticle is an exosome. In one embodiment, the cell is a mesenchymal stem cell and the microparticle is an exosome.

Conditional immortalisation may be achieved by introducing an immortalisation factor which is inactive unless the cell is supplied with an activating agent. Such an immortalisation factor may be a gene such as c-mycER. The c-MycER gene product is a fusion protein comprising a c-Myc variant fused to the ligand-binding domain of a mutant estrogen receptor. The conditionally-immortalised cells are typically cultured in the presence of the activating agent. For c-MycER conditionally-immortalised cells, the activating agent is 4-hydroxytamoxifen (4-OHT). Accordingly, the microparticles are typically isolated from conditionally-immortalised cells that are in the immortalised state at the time of microparticle isolation.

It has also been found that it is possible to alter the production of microparticles by conditionally-immortalised 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.

A second aspect of the invention provides a method of producing a microparticle, comprising isolating a microparticle from a conditionally-immortalised cell-conditioned medium, ~~optionally~~ wherein the cell is as defined above. In certain embodiments of this aspect:

the cell-conditioned medium may comprise one or more transforming growth factor-beta (TGF-β), interferon-gamma (IFN-γ) and/or tumour necrosis factor-alpha (TNF-α)~~components~~ which induce the release of microparticles by the stem cells into the medium;

the cells may be cultured under hypoxic conditions;

the cells may be co-cultured with a different cell type;

the cells may be cultured in a multi-compartment bioreactor; and/or

the cells may be stem cells that are partially-differentiated.

A further ~~aspect of the invention~~embodiment provides a method of producing a stem cell microparticle, typically a neural stem cell microparticle or a microparticle from another stem cell type (as detailed above). The method may comprise culturing the stem cells, typically conditionally-immortalised 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 embodiment, 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.

A further aspect of the invention provides a microparticle obtainable by a method aspect of the invention.

Another aspect of the invention provides a method of screening for an agent that alters the production of a microparticle by a conditionally-immortalised cell, comprising contacting a conditionally-immortalised cell with a candidate agent and observing whether the rate of

production of microparticles by the contacted conditionally-immortalised cell increases or decreases compared to a control.

A further aspect of the invention provides a kit for use in a method for producing a microparticle, comprising: (a) a medium; (b) a conditionally-immortalised cell as defined herein; (c) optionally the one or more transforming growth factor-beta (TGF-β), interferon-gamma (IFN-γ) and/or tumour necrosis factor-alpha (TNF-α) ascomponents 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 disclosed herein using the kit.

Further aspects and embodiments of the invention are defined below, and in the claims.
The invention also relates to the finding that neural stem cells contain microparticles that are therapeutically useful. The methods discussed herein with regard to the production of microparticles from neural stem cells may be applied to methods of producing microparticles from the other cell types described for the first aspect of the invention.

One aspect of the invention 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 conditionally-immortalised 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 embodiment, 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 embodiments, 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 embodiments, 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

CLAIMS

1. Use of a conditionally-immortalised cell to produce microparticles, wherein the conditionally-immortalised cell is:
    (i) a mesenchymal stem cell, optionally selected from a bone marrow derived stem cell, an endometrial regenerative cell, a mesenchymal progenitor cell, an adipose derived stem cell or a multipotent adult progenitor cell;
    (ii) a neural stem cell selected from a neurosphere initiating stem cell or an oligodendrocyte precursor cell;
    (iii) a haematopoietic stem cell, optionally a CD34+ cell and/or isolated from umbilical cord blood, or optionally a CD34+/CXCR4+ cell;
    (iv) a non-haematopoietic umbilical cord blood stem cell;
    (v) a very small embryonic like stem cell (VSEL);
    (vi) an induced pluripotent stem (iPS) cell;
    (vii) a fibroblast; or
    (viii) a dendritic cell.
2. The use according to claim 1, wherein the cell comprises c-mycER.
3. The use of claim 1 or claim 2, wherein the microparticle is an exosome, microvesicle, membrane particle, membrane vesicle, exosome-like vesicle, ectosome-like vesicle, ectosome or exovesicle.
4. The use according to any preceding claim, wherein the cell is as defined in claim 1(i), 1(ii), 1(iii), 1(iv), 1(v) or 1(vi) and is from a stem cell line.
5. The use of claim 4, wherein the stem cell line is grown in serum free medium.
6. The use 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 use of any preceding claim, wherein the microparticle comprises RNA.
8. The use of claim 7, wherein the RNA is mRNA and/or miRNA.
9. A method of producing a microparticle, comprising isolating a microparticle from a conditionally-immortalised cell-conditioned medium, wherein the cell is as defined in claim 1 or claim 2.

10. A method of producing a microparticle according to claim 9, wherein:

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

(ii) the cells were cultured under hypoxic conditions;

(iii) the cells were co-cultured with a different cell type;

(iv) the cells were cultured in a multi-compartment bioreactor; and/or

(v) the cells were stem cells that were partially-differentiated.

11. A kit for use in the method of claim 9 comprising: (a) a medium; (b) a conditionally-immortalised cell as defined in claim 1; (c) optionally one or more of transforming growth factor-beta (TGF-β), interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α); (d) optionally a microparticle obtainable by the method of claim 9 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 using the kit.

12. A method of screening for an agent that alters the rate of production of a microparticle by a conditionally-immortalised cell, comprising contacting a conditionally immortalised cell as defined in claim 1 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. Use of a conditionally-immortalised cell to produce microparticles.
2. The use according to claim 1, wherein the conditionally-immortalised cell is:

a mesenchymal stem cell, optionally selected from a bone marrow derived stem cell, an endometrial regenerative cell, a mesenchymal progenitor cell, an adipose derived stem cell or a multipotent adult progenitor cell;

a neural stem cell, optionally selected from a neurosphere initiating stem cell, or an oligodendrocyte precursor cell;

a haematopoietic stem cell, optionally a CD34+ cell and/or isolated from umbilical cord blood, or optionally a CD34+/CXCR4+ cell;

a non-haematopoietic umbilical cord blood stem cell;

a very small embryonic like stem cell (VSEL);

an induced pluripotent stem (iPS) cell;

a fibroblast; or

a dendritic cell.

3. The use according to claim 1 or claim 2, wherein the cell comprises c-mycER.
4. The use of any preceding claim, wherein the microparticle is an exosome, microvesicle, membrane particle, membrane vesicle, exosome-like vesicle, ectosome-like vesicle, ectosome or exovesicle.
5. The use according to any preceding claim, wherein the cell is from a stem cell line, optionally a neural stem cell line or a non-neural stem cell line.
6. The use of claim 5, wherein the stem cell line is grown in serum free medium.
7. The use of claim 6, wherein the stem cell line is a neural stem cell line, optionally CTX0E03 having ECACC Accession No. 04091601, STR0C05 having ECACC Accession No.04110301 and HPC0A07 having ECACC Accession No.04092302.
8. The use 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.

9. The use of any preceding claim, wherein the microparticle comprises RNA.
10. The use of claim 9, wherein the RNA is mRNA and/or miRNA.
11. The use of claim 10, wherein the microparticle comprises one, two, three or four of hsa-miR-1246, hsa-miR-4492, hsa-miR-4488 and hsa-miR-4532.
12. The use of any preceding claim, wherein the microparticle comprises 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.

13. The use of any preceding claim, wherein the microparticle comprises at least 10 of the proteins present in Table 19 or Table 21.

14. The use of any preceding claim, wherein the microparticle comprises at least one biological activity of a stem cell, a stem cell-conditioned medium, a neural stem cell or a neural stem cell-conditioned medium.

15. The use of claim 14, wherein the at least one biological activity is regenerative activity.

16. The microparticle produced by the use of any preceding claim, for use in therapy.

17. The microparticle of claim 16, wherein the therapy is regenerative therapy.

18. A method of producing a microparticle, comprising isolating a microparticle from a conditionally-immortalised cell-conditioned medium, optionally wherein the cell is as defined in claim 2 or claim 3.

19. A method of producing a microparticle according to claim 18, wherein:

(i) the cell-conditioned medium comprises one or more components which induce the release of microparticles by the stem cells into the medium;

(ii) the cells were cultured under hypoxic conditions;

(iii) the cells were co-cultured with a different cell type;

(iv) the cells were cultured in a multi-compartment bioreactor; and/or

(v) the cells were stem cells that were partially-differentiated.

20. A method according to claim 19(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).

21. A method according to claim 19(iii), or claim 20 when dependent upon claim 19(iii), wherein the different cell type is an endothelial cell.

22. A microparticle obtainable by the method of any of claims 19-21.

23. A composition comprising a microparticle according to claim 22 and a pharmaceutically acceptable excipient, carrier or diluent.

24. A kit for use in a method for producing the microparticle of claim 22 comprising: (a) a medium; (b) a conditionally-immortalised cell; (c) optionally the one or more components of claims 19 or 20; (d) optionally the microparticle of claim 22 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 claim 22 using the kit.

25. A method of screening for an agent that alters the rate of production of a microparticle by a conditionally-immortalised cell, comprising contacting a conditionally immortalised 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.