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 a first 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; and ii) collecting the microparticles that are produced by the cells. In a second aspect, the present invention refers to a microparticle obtainable by the method of any one of the present invention. In a third aspect, the present 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, the present 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.

~~A first aspect of the invention~~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 ~~embodiment~~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 ~~embodiments~~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 ~~embodiments~~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 ~~of the invention~~as disclosed herein is typically isolated or purified.

A ~~second aspect~~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,

neuropathy or any other disease or disorder that requires tissue regeneration, revascularisation or local anti-inflammatory action, including:

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

(ii) Lysosomal storage disorders;

(iii) Cardiovascular disorders, 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 disorders, such as Diabetes (I or II), rheumatoid arthritis, osteoarthritis, lupus, Crohn's disease, Inflammatory Bowel Disease, or Graft versus Host Disease;

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

(vii) Blindness-causing diseases of the retina, such as Age-related macular degeneration, Stargardt disease, diabetic retinopathy, retinitis pigmentosa; and

(viii) Demyelinating diseases, 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.

In one ~~embodiment~~example, the microparticle is an exosome and therapy is of a disease or condition requiring tissue replacement, regeneration or repair. In another ~~embodiment~~example, the microparticle is a microvesicle and the therapy is of a disease requiring angiogenesis or a neurological disease, disorder or deficit.

The therapy may also be a prophylactic therapy to induce tolerance, typically immunotolerance, in a host that is subsequently, concurrently or simultaneously to receive the stem cells from which the microparticle is derived. The administration of one or more doses of microparticles ~~of the invention~~disclosed herein to a patient, prior to or concurrent with administration of a stem cell therapy, can be used to reduce the risk of an adverse immune response, i.e. "rejection", of the stem cell therapy.

~~A third aspect of the invention~~Another example provides the use of a neural stem cell microparticle in the manufacture of a medicament for the treatment of a disease.

~~A fourth aspect~~A further example of the invention provides 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 ~~embodiment~~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.

~~A fifth aspect of the invention~~A further example provides a microparticle obtainable by a method ~~according to the fourth aspect of the invention~~disclosed herein.

~~A sixth aspect of the invention~~Another example provides a composition comprising a neural stem cell microparticle and a pharmaceutically acceptable excipient, carrier or diluent.

~~A seventh aspect of the invention~~A further example 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.

~~An eighth aspect of the invention~~Another example provides 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.

~~A ninth aspect of the invention~~A yet further example 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

pharmaceutical composition, comprising a pharmaceutically-acceptable carrier, diluent, vehicle and/or excipient. The pharmaceutical composition is suitable for use in therapy, typically in the same therapies as the microparticles ~~of the invention~~disclosed herein, as noted above.

Brief Description of the Drawings

Figure 1 depicts electron micrographs of CTX0E03 conditionally-immortalised neural stem cells producing microparticles. Panels A-E show intracellular multivesicular bodies (MVBs) containing exosomes between 30nm and 50nm in diameter and Panel F shows microvesicles >100nm in diameter released from neural stem cells through a process of budding at the cell membrane.

Figure 2 is an outline protocol for the identification, characterisation and production of microparticles from stem cells.

Figure 3 shows Human angiogenesis ELISA strip optical density read out performed on CTX0E03 conditioned and un-conditioned medium.

Figure 4A shows the amount of protein (measured by BCA assay) extracted from 15ml of media containing microparticles purified from the Integra system compared to normal culture conditions (3 days T175). Figure 4B shows the FACS detection (at 2ug/ml, 1:250) of (i) CD63 in Integra cultured CTX0E03 exosomes (top left panel) and microvesicles (top right panel) and (ii) CD81 in Integra cultured CTX0E03 exosomes (bottom left panel) and microvesicles (bottom right panel).

Figure 5 shows the amount of isolated total RNA measured at 260/280nm extracted from 15ml of media containing microparticles purified by filtration from the Integra system compared to normal culture conditions (3 days T175).

Figure 6A shows the results of a wound closure/scratch assay representing the migration activity of normal human dermal fibroblasts (NHDF) cultured in CTX0E03 conditioned media or upon the addition of purified CTX0E03 exosomes. Figure 6B shows the results of a scratch assay after 72 hours, comparing the effect of 10µg CTX0E03 exosomes to basal conditions (without exosomes). Figure 6C shows the % of healed areas for basal conditions, 2µg/ml exosomes, 6 µg/ml exosomes, 20 µg/ml exosomes and an LSGS (low serum growth supplement) positive control. The top panel of Figure 6C shows exosomes isolated from CTX0E03 cells cultured for 2 weeks in the Integra Celline system and the bottom panel of Figure 6C shows exosomes isolated from CTX0E03 cells cultured for 6 weeks in the Integra

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 ~~administering~~ administration of stem cells together with the microparticles.