

HYBRID COMPOSITION

Field of the Invention

The invention relates to a novel chemical composition comprising living cells combined with biocompatible fibres and biocompatible components which are optimally chemically formulated to repair damaged human or animal tissue.

Background to the Invention

A variety of different cell types are used in repairing damaged tissue. Once administered to the patient, the cells typically exert their therapeutic effects through paracrine signalling and by promoting survival, repair and regeneration of the neighbouring cells in the damaged tissue.

The repair of damaged cells by therapeutic cells is often inefficient because (1) the cells can only access and repair the surface of the damaged tissue, but cannot reach deeper parts of the damaged tissue, (2) the cells remain in the optimal location for repair for a limited time period and (3) the local microenvironment in which the cells are placed is not optimal to promote repair and regeneration. These limitations often result in a sub-optimal or partial repair of the damaged tissue.

Summary of the Invention

The inventors have surprisingly found that a unique hybrid composition of living cells, biocompatible fibres and biocompatible components is more effective at repairing damaged tissues. For instance, the biocompatible fibres and components are capable of providing the cells with access to the optimal location to effect optimal repair of damaged tissue. The cells may, for example, reach deeper parts of the damaged tissue. The biocompatible fibres and components are also capable of retaining the cells at the optimal location to effect optimal repair of damaged tissue. The composition also creates its own optimal microenvironment for optimal repair and regeneration.

The invention therefore provides a hybrid composition, which comprises:

(a) one or more biocompatible fibres;

(b) one or more therapeutic cells; and

(c) one or more biocompatible components which (i) attach the one or more therapeutic cells to the one or more fibres and/or embed the one or more therapeutic cells and the one or more fibres or (ii) are capable of attaching the composition to a tissue.

The invention also provides:

- a liposome composition comprising one or more hybrid compositions of the invention contained within one or more liposomes;
- a microbubble composition comprising one or more hybrid compositions of the invention contained within one or more microbubbles;
- 5 - a method of producing a hybrid composition of the invention, comprising (a) providing one or more biocompatible fibres, one or more therapeutic cells and one or more biocompatible components and (b) attaching the one or more therapeutic cells to the one or more fibres using one or more biocompatible components and/or embedding the one or more therapeutic cells and the one or more fibres in one or more biocompatible components or combining the one or more fibres, the one or more
10 cells and the one or more components and thereby producing a hybrid composition of the invention;
- a method of repairing a damaged tissue in a patient, comprising contacting the damaged tissue with a hybrid composition of the invention, a liposome composition of the invention or a microbubble composition of the invention, wherein the composition comprises a therapeutically effective number of cells, and thereby treating the damaged tissue in the patient; or
- 15 - a hybrid composition of the invention, a liposome composition of the invention or a microbubble composition of the invention for use in a method of repairing a damaged tissue in a patient.

Brief Description of the Figures

20 Figure 1 shows the general structure of a hybrid composition of the invention in which the therapeutic cells are attached to the one or more fibres using a biocompatible component.

Figure 2 shows the general structure of a hybrid composition of the invention in which the therapeutic cells are embedded in the biocompatible component.

Figure 3 shows the preferred structures of hybrid compositions of the type shown in Figure 1.
25 In each case, the therapeutic cells are human mesenchymal stem cells (MSCs) and the one or more fibres comprise poly(D,L-lactide-co-glycolide) (PLGA). The biocompatible component comprises fibrin, integrin or adherin.

Figure 4 shows the preferred structures of hybrid compositions of the type shown in Figure 2.
In each case, the therapeutic cells are human mesenchymal stem cells (MSCs) and the one or more
30 fibres comprise poly(D,L-lactide-co-glycolide) (PLGA). The biocompatible component comprises fibrin, integrin or cadherin.

Figure 5 shows the preferred structures of hybrid compositions of the type shown in Figure 1.
In each case, the therapeutic cells are mesenchymal precursor cells (MPCs) and the one or more fibres

comprise poly(D,L-lactide-co-glycolide) (PLGA). The biocompatible component comprises fibrin, integrin or cadherin.

Figure 6 shows the preferred structures of hybrid compositions of the type shown in Figure 2. In each case, the therapeutic cells are mesenchymal precursor cells (MPCs) and the one or more fibres
5 comprise poly(D,L-lactide-co-glycolide) (PLGA). The biocompatible component comprises fibrin, integrin or cadherin.

Detailed Description of the Invention

It is to be understood that different applications of the disclosed products and methods may be
10 tailored to the specific needs in the art. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

In addition, as used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the content clearly dictates otherwise. Thus, for example,
15 reference to “a cell” includes “cells”, reference to “a tissue” includes two or more such tissues, reference to “a patient” includes two or more such patients, and the like.

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

Composition of the invention

The invention provides a hybrid composition. The composition is a hybrid because it comprises living cells and non-living fibres and components.

The composition is suitable for repairing damaged tissue. The composition may be implanted or injected into damaged tissue. The composition may be adhered to or anchored to damaged tissue.
25 This is discussed in more detail below with reference to the therapeutic embodiments of the invention.

Biocompatible fibres

The composition of the invention comprises one or more biocompatible fibres. A fibre is biocompatible if it does not cause any adverse reactions or side effects when contacted with a damaged
30 tissue.

Any number of biocompatible fibres may be present in the composition. The composition may comprise only one fibre. The composition typically comprises more than one fibre, such at least 2, at

least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 100, at least 200, at least 500 fibres, at least 1000 fibres or even more fibres.

Suitable biocompatible fibres are known in the art. The one or more biocompatible fibres may be natural or synthetic. Preferred biocompatible fibres include, but are not limited to, cellulose fibres, collagen fibres, collagen-glycosaminoglycan fibres, gelatin fibres, silk fibroin fibres, one or more fibrin fibres, chitosan fibres, starch fibres, alginate fibres, hyaluronan fibres, poloxamer fibres or a combination thereof. The glycosaminoglycan is preferably chondroitin. The cellulose is preferably carboxymethylcellulose, hydroxypropylmethylcellulose or methylcellulose. The poloxamer is preferably pluronic acid, optionally Pluronic F-127.

If more than one fibre is present in the composition, the population of fibres may be homogenous. In other words, all of the fibres in the population may be the same type of fibre, e.g. cellulose fibres. Alternatively, the population of fibres may be heterogeneous. In other words, the population of fibres may contain different types of fibre, such cellulose fibres and collagen fibres.

The one or more fibres may be any length. The one or more fibres are preferably approximately the same length as the depth of the damage in the tissue which is to be treated using the composition. The length of one or more fibres is preferably designed such that the composition can penetrate a damaged tissue to a prescribed depth. The one or more fibres may be any length. The lower limit of the length of the one or more fibres is typically determined by the diameter of the one or more therapeutic cells. Suitable lengths include, but are not limited to, at least 1µm in length, at least 10µm in length, at least 100µm in length, at least 500µm in length, at least 1mm in length, at least 10mm (1cm) in length, at least 100mm (10cm) in length, at least 500mm (50cm) in length or at least 1000mm (100cm or 1m) in length. The one or more fibres may be even longer. For instance, the one or more fibres may be up to 5m or 10m in length, for instance if being used to repair damage along the human intestinal tract, or even longer if being used in larger animals, such as horses. The length of the one or more fibres is typically determined by their intended use and/or their ability to be manipulated, for instance by a surgeon, by a robot or via some other means, such as magnetically.

The one or more fibres may be charged. The one or more fibres are preferably positively-charged. The one or more fibres are preferably negatively-charged.

The one or more fibres may be magnetic. The one or more fibres may be modified to include one or more magnetic atoms or groups. This allows magnetic targeting of the composition. The magnetic atoms or groups may be paramagnetic or superparamagnetic. Suitable atoms or groups include, but are not limited to, gold atoms, iron atoms, cobalt atoms, nickel atoms and a metal chelating groups, such as nitrilotriacetic acid, containing any of these atoms. The metal chelating group may, for

instance, comprise a group selected from -C(=O)O-, -C-O-C-, -C(=O), -NH-, -C(=O)-NH, -C(=O)-CH₂-I, -S(=O)₂- and -S-.

Biocompatible components

5 The composition also comprises one or more biocompatible components. The one or more biocompatible components (i) attach the one or more therapeutic cells to the one or more fibres and/or embed the one or more therapeutic cells and the one or more fibres and/or (ii) are capable of attaching the composition to a tissue. The one or more biocompatible components may (a) attach the one or more therapeutic cells to the one or more fibres, (b) embed the one or more therapeutic cells and the one or
10 more fibres, (c) be capable of attaching the composition to a tissue, (d) attach the one or more therapeutic cells to the one or more fibres and embed the one or more therapeutic cells and the one or more fibres, (e) attach the one or more therapeutic cells to the one or more fibres and be capable of attaching the composition to a tissue, (f) embed the one or more therapeutic cells and the one or more fibres and be capable of attaching the composition to a tissue or (g) attach the one or more therapeutic
15 cells to the one or more fibres, embed the one or more therapeutic cells and the one or more fibres and be capable of attaching the composition to a tissue.

A component is biocompatible if it does not cause any adverse reactions or side effects when contacted with a damaged tissue.

Any number of biocompatible components may be present in the composition. The
20 composition typically comprises only one component or two components. The composition may comprise more than two components, such as at least 3, at least 5, at least 10, at least 20, at least 30, at least 40, at least 50 components or even more components.

The one or more biocompatible components preferably comprise a biocompatible adhesive which attaches the one or more therapeutic cells to the one or more fibres. The biocompatible adhesive
25 may attach the one or more therapeutic cells (a) on the surface of the one or more fibres, (b) within the one or more fibres or (c) both on the surface of and within the one or more fibres.

The biocompatible adhesive may be natural or synthetic. Suitable biocompatible adhesives are known in the art. Suitable adhesives include, but are not limited to, fibrin, fibrin gel, integrin, integrin gel, cadherin and cadherin gel.

30 The one or more biocompatible components preferably comprise a biocompatible gel which embeds the one or more therapeutic cells and the one or more fibres. Suitable biocompatible gels are known in the art. The biocompatible gel may be natural or synthetic. Preferred biocompatible gels

include, but are not limited to, a cellulose gel, a collagen gel, a gelatin gel, a fibrin gel, a chitosan gel, a starch gel, an alginate gel, a hyaluronan gel, an agarose gel, a poloxamer gel or a combination thereof.

The cellulose gel may be formed from any of the celluloses discussed above. The cellulose polymer concentration is preferably from about 1.5% (w/w) to about 4.0% (w/w), such as from about 2.0% (w/w) to about 3.0% (w/w). The cellulose polymer preferably has a molecular weight of from about 450,000 to about 4,000,000, such as from about 500,000 to about 3,500,000, from about 500,000 to about 3,000,000 or from about 750,000 to about 2,500,000 or from about 1000,000 to about 2,000,000.

The poloxamer gel is preferably a pluronic acid gel, optionally a Pluronic F-127 gel.

The adhesive and/or gel preferably has a viscosity in the range of 1000 to 500,000 mPa•s (cps) at room temperature, such as from about 1500 to about 450,000 mPa•s at room temperature, from about 2000 to about 400,000 mPa•s at room temperature, from about 2500 to about 350,000 mPa•s at room temperature, from about 5000 to about 300,000 mPa•s at room temperature, from about 10,000 to about 250,000 mPa•s at room temperature, from about 50,000 to about 200,000 mPa•s at room temperature or from about 50,000 to about 150,000 mPa•s at room temperature.

Viscosity is a measure of the resistance of the adhesive and/or gel to being deformed by either shear stress or tensile stress. Viscosity can be measured using any method known in the art. Suitable methods include, but are not limited to, using a viscometer or a rheometer.

Room temperature is typically from about 18 °C to about 25 °C, such as from about 19 °C to about 24 °C or from about 20 °C to about 23 °C or from about 21 °C to about 22 °C. Room temperature is preferably any of 18 °C, 19 °C, 20 °C, 21 °C, 22 °C, 23 °C, 24 °C and 25 °C. Viscosity is most preferably measured at 25 °C.

The one or more biocompatible components preferably comprises a biocompatible adhesive which attaches the one or more therapeutic cells to the one or more fibres and a biocompatible gel which embeds the one or more therapeutic cells and the one or more fibres. For instance, the composition may comprise a fibrin gel which attaches the one or more therapeutic cells to the one or more fibres and a cellulose gel which embeds the one or more therapeutic cells and the one or more fibres.

In any of the embodiments discussed above, the biocompatible adhesive and/or the biocompatible gel preferably comprises platelet lysate. For instance, the adhesive and/or the gel may be a platelet lysate gel. Platelet lysate refers to the combination of natural growth factors contained in platelets that has been released through lysing those platelets. Lysis can be accomplished through chemical means (i.e. CaCl_2), osmotic means (use of distilled H_2O) or through freezing/thawing

procedures. Platelet lysate can be derived from whole blood as described in U.S. Pat. No. 5,198,357. Platelet lysate is preferably prepared as described in PCT/GB12/052911 (published as WO 2013/076507). For instance, it may be prepared by subjecting a population of platelets to at least one freeze-thaw cycle, wherein the freeze portion of each cycle is carried out at a temperature lower than or equal to - 78 °C.

The adhesive and/or gel preferably comprises (a) platelet lysate, (b) at least one pharmaceutically acceptable polymer and (c) at least one pharmaceutically acceptable positively charged chemical species selected from the group consisting of lysine, arginine, histidine, aspartic acid, glutamic acid, alanine, methionine, proline, serine, asparagine, cysteine, polyamino acids, protamine, aminoguanidine, zinc ions and magnesium ions, wherein the composition is an aqueous gel having a viscosity in the range of 1000 to 500,000 mPa·s (cps) at room temperature. The pharmaceutically acceptable polymer is preferably cellulose or a poloxamer. It may be any of the celluloses and poloxamers discussed above.

The platelet lysate is preferably human platelet lysate. Platelet lysate is discussed in more detail below.

Therapeutic cells

The composition also comprises one or more therapeutic cells. Therapeutic cells are cells which are capable of having a therapeutic effect. Therapeutic cells are typically living cells.

Therapeutic cells are typically cells which are capable of repairing damaged tissue. The one or more therapeutic cells are preferably autologous. In other words, the one or more cells are preferably derived from the patient into which the cells will be administered to repair damaged tissue. Alternatively, the one or more cells are preferably allogeneic. In other words, the cells are preferably derived from a patient that is immunologically compatible with the patient into which the cells will be administered to repair damaged tissue. The one or more cells may be semi-allogeneic. Semi-allogeneic populations are typically produced from two or more patients that are immunologically compatible with the patient into which the cells will be administered. In other words, all of the one or more cells are preferably genetically identical with the patient into which they will be administered or sufficiently genetically identical that the cells are immunologically compatible with the patient into which they will be administered.

Any number of cells may be present in the composition. The composition may comprise only one cell. For instance, the composition may comprise one cell and one fibre.

The composition typically comprises more than one cell, such at least 2, at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 100, at least 200, at least 500, at least 1000, at least 2000, at least 5000, at least 10000, at least 50000, at least 100000, at least 5×10^5 , at least 1×10^6 , at least 2×10^6 , at least 5×10^6 , at least 1×10^7 , at least 2×10^7 , at least 5×10^7 , at least 1×10^8 or at least 2×10^8 cells. In some instances, the composition may comprise at least 1.0×10^7 , at least 1.0×10^8 , at least 1.0×10^9 , at least 1.0×10^{10} , at least 1.0×10^{11} or at least 1.0×10^{12} cells or even more cells.

If more than one cell is present in the composition, the population of cells may be homogenous. In other words, all of the cells in the population may be the same type of cell, e.g. mesenchymal stem cells (MSCs). Alternatively, the population of cells may be heterogeneous. In other words, the population of cells may contain different types of cells, such MSCs and progenitor cells of mesodermal lineage (PMLs).

The one or more therapeutic cells are preferably one or more progenitor cells of mesodermal lineage (PMLs). PMLs are disclosed in PCT/GB2012/051600 (published as WO 2013/005053). Any of the cells disclosed therein may be used. The PML expresses detectable levels of CD29, CD44, CD73, CD90, CD105 and CD271, but does not express detectable levels of CD14, CD34 and CD45.

The PMLs may advantageously be used to repair damaged tissues in patients. The PMLs are capable of efficiently exerting anti-inflammatory effects in the tissue. The anti-inflammatory effects of the PMLs promote survival, repair and regeneration of the neighbouring cells in the damaged tissue. The cells are also able to exert paracrine effects such as the secretion of angiogenic, chemotactic and anti-apoptotic factors.

As discussed in more detail below, the PMLs are produced from mononuclear cells (MCs), such as peripheral MCs, taken from an individual, such as a human individual. Since the PMLs are produced from MCs, they may be produced easily (such as from peripheral blood) and may be autologous for the patient to be treated and thereby avoid the risk of immunological rejection by the patient.

It is possible, in principle, to produce an unlimited number of PMLs from a single individual, since various samples of MCs (i.e. various samples of blood) may be obtained. It is certainly possible to produce very large numbers of PMLs from a single individual. The PMLs of the invention can therefore be made in large numbers.

The PMLs are produced in clinically relevant conditions, for instance in the absence of trace amounts of endotoxins and other environmental contaminants, as well as animal products such as fetal calf serum. This makes the PMLs particularly suitable for administration to patients.

Since the PMLs are produced from MCs, they are substantially homologous and may be autologous. They also avoid donor-to-donor variation, which frequently occurs with mesenchymal stem cells (MSCs). Numerous populations of PMLs can be produced from a single sample taken from the patient before any other therapy, such as chemotherapy or radiotherapy, has begun. Therefore, the PMLs can avoid any of the detrimental effects of those treatments.

The PMLs can be made quickly. PMLs can be produced from MCs in less than 30 days, such as in about 22 days.

The production of PMLs from MCs avoids the moral and ethical implications involved with using mesenchymal stem cells (MSCs) derived from human embryonic stem cells (hESCs).

The PMLs are typically produced from human MCs. The PMLs are therefore typically human. Alternatively, the PML cells may be produced from MCs from other animals or mammals, for instance from commercially farmed animals, such as horses, cattle, sheep or pigs, from laboratory animals, such as mice or rats, or from pets, such as cats, dogs, rabbits or guinea pigs.

The PMLs can be identified as progenitor cells of mesodermal lineage using standard methods known in the art, including expression of lineage restricted markers, structural and functional characteristics. The PMLs will express detectable levels of cell surface markers known to be characteristic of progenitor cells of mesodermal lineage. In particular, in addition to the markers discussed in more detail below, the PMLs may express α -smooth muscle actin, collagen type I α -chain, GATA6, Mohawk, and vimentin (Sági B et al Stem Cells Dev. 2012 Mar 20; 21(5):814-28).

The PMLs are capable of successfully completing differentiation assays *in vitro* to confirm that they are of mesodermal lineage. Such assays include, but are not limited to, adipogenic differentiation assays, osteogenic differentiation assays and neurogenic differentiation assays (Zaim M et al Ann Hematol. 2012 Aug;91(8):1175-86).

The PMLs are not stem cells. In particular, they are not mesenchymal stem cells (MSCs). They are terminally differentiated. Although they can be forced under the right conditions *in vitro* to differentiating, for instance into cartilage or bone cells, they do not differentiate *in vivo*. The PMLs have their effects by exerting paracrine signalling in the damaged tissue. In particular, the PMLs are preferably capable of inducing anti-inflammatory effects in the damaged tissue. This is discussed in more detail below.

The PMLs are typically characterised by a spindle-shaped morphology. The PMLs are typically fibroblast-like, i.e. they have a small cell body with a few cell processes that are long and thin. The cells are typically from about 10 to about 20 μ m in diameter.

The PMLs are distinguished from other cells via their marker expression pattern. The PMLs express detectable levels of CD29, CD44, CD73, CD90, CD105 and CD271. The PMLs may overexpress one or more of, such as all of, CD29, CD44, CD73, CD90, CD105 and CD271. The PMLs overexpress one or more of CD29, CD44, CD73, CD90, CD105 and CD271 if they express more than
5 other PMLs and/or MSCs. The PMLs do not express detectable levels of CD14, CD34 and CD45. The PMLs preferably express CD62E (E-selectin) and /or CD62P (P-selectin).

Standard methods known in the art may be used to determine the detectable expression, low expression or lack thereof of the various markers discussed above (and below). Suitable methods include, but are not limited to, immunocytochemistry, immunoassays, flow cytometry, such as
10 fluorescence activated cells sorting (FACS), and polymerase chain reaction (PCR), such as reverse transcription PCR (RT-PCR). Suitable immunoassays include, but are not limited to, Western blotting, enzyme-linked immunoassays (ELISA), enzyme-linked immunosorbent spot assays (ELISPOT assays), enzyme multiplied immunoassay techniques, radioallergosorbent (RAST) tests, radioimmunoassays, radiobinding assays and immunofluorescence. Western blotting, ELISAs and RT-PCR are all
15 quantitative and so can be used to measure the level of expression of the various markers if present. The use of FACS is preferred. Antibodies and fluorescently-labelled antibodies for all of the various markers discussed herein are commercially-available.

The one or more therapeutic cells are preferably one or more immuno-modulatory progenitor (IMP) cells as disclosed in the UK Application being filed concurrently with this application (CTL
20 Ref. IMP1). IMP cells express detectable levels of MIC A/B, CD304 (Neuropilin 1), CD178 (FAS ligand), CD289 (Toll-like receptor 9), CD363 (Sphingosine-1-phosphate receptor 1), CD99, CD181 (C-X-C chemokine receptor type 1; CXCR1), epidermal growth factor receptor (EGF-R), CXCR2 and CD126.

The IMP cells may advantageously be used to repair damaged tissues in patients. The IMP
25 cells are capable of exerting anti-inflammatory effects in the tissue. The anti-inflammatory effects of the IMP cells promote survival, repair and regeneration of the neighbouring cells in the damaged tissue. The cells are also able to exert paracrine effects such as the secretion of angiogenic, chemotactic and anti-apoptotic factors.

The IMP cells are typically produced from human MCs. The IMP cells of the invention are
30 therefore typically human. Alternatively, the IMP cells may be produced from MCs from other animals or mammals, for instance from commercially farmed animals, such as horses, cattle, sheep or pigs, from laboratory animals, such as mice or rats, or from pets, such as cats, dogs, rabbits or guinea pigs.

The IMP cells are also produced from mononuclear cells (MCs), such as peripheral MCs, taken from an individual, such as a human individual. Hence, they have the same advantages as PMLs discussed above. The IMP cells are capable of successfully completing differentiation assays *in vitro* to confirm that they are of mesodermal lineage. Such assays include, but are not limited to, adipogenic differentiation assays, osteogenic differentiation assays and neurogenic differentiation assays (Zaim M et al Ann Hematol. 2012 Aug;91(8):1175-86).

The IMP cells are not stem cells. In particular, they are not MSCs. They are terminally differentiated. Although they can be forced under the right conditions *in vitro* to differentiating, for instance into cartilage or bone cells, they typically do not differentiate *in vivo*. The IMP cells of the invention have their effects by exerting paracrine signalling in the damaged tissue. In particular, the IMP cells are preferably capable of inducing anti-inflammatory effects in the damaged tissue. This is discussed in more detail below.

The IMP cells are typically characterised by a spindle-shaped morphology. The IMP cells are typically fibroblast-like, i.e. they have a small cell body with a few cell processes that are long and thin. The cells are typically from about 10 to about 20 µm in diameter.

The IMP cells of the invention are distinguished from known cells, including MSCs, via their marker expression pattern. The IMP cells express detectable levels of MIC A/B, CD304 (Neuropilin 1), CD178 (FAS ligand), CD289 (Toll-like receptor 9), CD363 (Sphingosine-1-phosphate receptor 1), CD99, CD181 (C-X-C chemokine receptor type 1; CXCR1), epidermal growth factor receptor (EGF-R), CXCR2 and CD126. The IMP cells preferably express an increased amount of these markers compared with MSCs. This can be determined by comparing the expression level/amount of the markers in an IMP of the invention with the expression level/amount in an MSC using the same technique under the same conditions. Suitable MSCs are commercially available. The MSC used for comparison is preferably a human MSC. Human MSCs are commercially available from Mesoblast® Ltd, Osiris Therapeutics® Inc. or Lonza®. The human MSC is preferably obtained from Lonza®. Such cells were used for the comparison in the Example. The MSC may be derived from any of the animals or mammals discussed above.

The IMP cells preferably express an increased amount of one or more of MIC A/B, CD304 (Neuropilin 1), CD178 (FAS ligand), CD289 (Toll-like receptor 9), CD363 (Sphingosine-1-phosphate receptor 1), CD99, CD181 (C-X-C chemokine receptor type 1; CXCR1), epidermal growth factor receptor (EGF-R), CXCR2 and CD126 compared with a MSC. The IMP cells preferably express an increased amount of all of the ten markers compared with a MSC.

Any of the methods disclosed above may be used to determine the detectable expression or increased expression of these markers. The expression or increased expression of any of the markers disclosed herein is preferably done using high-throughput FACS (HT-FACS).

The IMP cells preferably demonstrate an antibody mean fluorescence intensity (MFI) of at least 330, such as at least 350 or at least 400, for MIC A/B, an MFI of at least 210, such as at least 250 or at least 300, for CD304 (Neuropilin 1), an MFI of at least 221, such as at least 250 or at least 300, for CD178 (FAS ligand), an MFI of at least 186, such as at least 200 or at least 250, for CD289 (Toll-like receptor 9), an MFI of at least 181, such as at least 200 or at least 250, for CD363 (Sphingosine-1-phosphate receptor 1), an MFI of at least 184, such as at least 200 or at least 250, for CD99, an MFI of at least 300, such as at least 350 or at least 400, for CD181 (C-X-C chemokine receptor type 1; CXCR1), an MFI of at least 173, such as at least 200 or at least 250, for epidermal growth factor receptor (EGF-R), an MFI of at least 236, such as at least 250 or at least 300, for CXCR2 and an MFI of at least 160, such as at least 200 or at least 250, for CD126. Mean fluorescent intensity (MFI) is a measure of intensity, time average energy flux measured in watts per square metre. It is an SI unit. The MFI for each marker is typically measured using HT-FACS. The MFI for each marker is preferably measured using HT-FACS as described in the Example of the UK Application being filed concurrently with this application (CTL Ref. IMP1).

In addition to the ten markers specified above, the IMP cells typically express detectable levels of one or more of the other markers shown in Table 1 in the Example of the UK Application being filed concurrently with this application (CTL Ref. IMP1). The IMP cells may express detectable levels of any number and combination of those markers.

The IMP cells preferably express detectable levels of one or more of CD267, CD47, CD51/CD61, CD49f, CD49d, CD146, CD340, Notch2, CD49b, CD63, CD58, CD44, CD49c, CD105, CD166, HLA-ABC, CD13, CD29, CD49e, CD73, CD81, CD90, CD98, CD147, CD151 and CD276. The IMP cells more preferably express detectable levels of one or more of CD10, CD111, CD267, CD47, CD273, CD51/CD61, CD49f, CD49d, CD146, CD55, CD340, CD91, Notch2, CD175s, CD82, CD49b, CD95, CD63, CD245, CD58, CD108, B2-microglobulin, CD155, CD298, CD44, CD49c, CD105, CD166, CD230, HLA-ABC, CD13, CD29, CD49e, CD59, CD73, CD81, CD90, CD98, CD147, CD151 and CD276. The IMP cells may express detectable levels of any number and combination of these markers. The IMP cells preferably express detectable levels of all of these markers.

The IMP cells preferably express detectable levels of one or more of CD156b, CD61, CD202b, CD130, CD148, CD288, CD337, SSEA-4, CD349 and CD140b. The IMP cells more preferably

express detectable levels of one or more of CD156b, CD61, CD202b, CD130, CD148, CD288, CD337, SSEA-4, CD349, CD140b, CD10, CD111, CD267, CD47, CD273, CD51/CD61, CD49f, CD49d, CD146, CD55, CD340, CD91, Notch2, CD175s, CD82, CD49b, CD95, CD63, CD245, CD58, CD108, B2-microglobulin, CD155, CD298, CD44, CD49c, CD105, CD166, CD230, HLA-ABC, CD13, CD29, CD49e, CD59, CD73, CD81, CD90, CD98, CD147, CD151 and CD276. The IMP cells may express detectable levels of any number and combination of these markers. The IMP cells preferably express detectable levels of all of these markers.

The IMP cells preferably express detectable levels of one or more of CD72, CD133, CD192, CD207, CD144, CD41b, FMC7, CD75, CD3e, CD37, CD158a, CD172b, CD282, CD100, CD94, CD39, CD66b, CD158b, CD40, CD35, CD15, PAC-1, CLIP, CD48, CD278, CD5, CD103, CD209, CD3, CD197, HLA-DM, CD20, CD74, CD87, CD129, CDw329, CD57, CD163, TPBG, CD206, CD243 (BD), CD19, CD8, CD52, CD184, CD107b, CD138, CD7, CD50, HLA-DR, CD158e2, CD64, DCIR, CD45, CLA, CD38, CD45RB, CD34, CD101, CD2, CD41a, CD69, CD136, CD62P, TCR alpha beta, CD16b, CD1a, ITGB7, CD154, CD70, CDw218a, CD137, CD43, CD27, CD62L, CD30, CD36, CD150, CD66, CD212, CD177, CD142, CD167, CD352, CD42a, CD336, CD244, CD23, CD45RO, CD229, CD200, CD22, CDH6, CD28, CD18, CD21, CD335, CD131, CD32, CD157, CD165, CD107a, CD1b, CD332, CD180, CD65 and CD24. The IMP cells may express detectable levels of any number and combination of these markers. The IMP cells preferably express detectable levels of all of these markers.

The one or more IMP cells may be part of any of the populations disclosed in the UK Application being filed concurrently with this application (CTL Ref. IMP1).

The IMP cells are preferably capable of adhering to a specific, damaged tissue in a patient. Adherence and adhesion assay are known in the art (Humphries, *Methods Mol Biol.* 2009;522:203-10).

The IMP cells are preferably capable of proliferating in a specific, damaged tissue in a patient. Cell proliferation assays are well known in the art. Such assays are commercially available, for instance from Life Technologies®.

The IMP cells are preferably capable of promoting angiogenesis in a specific, damaged tissue in a patient. Angiogenesis assays are known in the art (Auerback *et al.*, *Clin Chem.* 2003 Jan;49(1):32-40).

The IMP cells are preferably capable of having anti-inflammatory effects in a damaged tissue of a patient. The ability of the IMP cells of the invention to have anti-inflammatory effects may also be measured using standard assays known in the art. Suitable methods include, but are not limited to, enzyme-linked immunosorbent assays (ELISAs) for the secretion of cytokines, enhanced mixed

leukocyte reactions and up-regulation of co-stimulatory molecules and maturation markers, measured by flow cytometry. Specific methods that may be used are disclosed in the Example. The cytokines measured are typically interleukins, such as interleukin-8 (IL-8), selectins, adhesion molecules, such as Intercellular Adhesion Molecule-1 (ICAM-1), and chemoattractant proteins, such as monocyte
 5 chemotactic protein-1 (MCP-1) and tumour necrosis factor alpha (TNF-alpha). Assays for these cytokines are commercially-available. Anti-inflammatory factors are preferably detected and measured using the Luminex® assay described in the Examples. Such assays are commercially available from Life Technologies®.

The IMP cells preferably secrete detectable levels of one or more of interleukin-6 (IL-6), IL-8,
 10 C-X-C motif chemokine 10 (CXCL10; interferon gamma-induced protein 10; IP-10), Chemokine (C-C motif) ligand 2 (CCL2; monocyte chemotactic protein-1; MCP-1) and Chemokine (C-C motif) ligand 5 (CCL5; regulated on activation, normal T cell expressed and secreted; RANTES). The IMP cells may secrete any number and combination of these factors. The IMP cells preferably secrete all of these markers.

15 The IMP cells preferably secrete an increased amount of one or more of IL-6, IL-8, IP-10, MCP-1 and RANTES compared with a MSC. The IMP cells may secrete an increased amount of any number and combination of these factors. The IMP cells preferably secrete an increased amount of all of these markers.

The IMP cells preferably secrete a decreased amount of interleukin-10 (IL-10) and/or IL-12
 20 compared with a mesenchymal stem cell MSC. IL-10 and IL-12 are pro-inflammatory cytokines.

The IMP cells will express a variety of different other markers over and above those discussed above. Some of these will assist the IMP cells with their ability to have anti-inflammatory effects in a damaged tissue. Any of the IMP cells of the invention may further express detectable levels of one or more of (i) insulin-like growth factor-1 (IGF-1), (ii) IGF-1 receptor; (iii) C-C chemokine receptor type
 25 1 (CCR1), (iv) stromal cell-derived factor-1 (SDF-1), (v) hypoxia-inducible factor-1 alpha (HIF-1 alpha), (vi) Akt1 and (vii) hepatocyte growth factor (HGF) and/or granulocyte colony-stimulating factor (G-CSF).

IGF-1 receptors promote migration capacity towards an IGF-1 gradient. One of the mechanisms by which IGF-1 increases migration is by up-regulating CXCR4 on the surface of the
 30 cells, which makes them more sensitive to SDF-1 signaling. This is discussed above.

CCR1 is the receptor for CCL7 (previously known as MCP3) increases homing and engraftment capacity of MSCs (and so would be expected to have the same effect for the IMP cells of

the invention) and can increase the capillary density in injured myocardium through paracrine signalling.

HIF-1 alpha activates pathways that increase oxygen delivery and promote adaptive pro-survival responses. Among the many target genes of HIF-1 alpha are erythropoietin (EPO), endothelin and VEGF (with its receptor Flk-1). IMP cells that express or express an increased amount of HIF-1alpha will have upregulated expression of paracrine stimuli of for example several vasculogenic growth factors that may promote a more therapeutic subtype. As described in more detail below, the IMP cells of the invention can be preconditioned into a more therapeutic subtype by culturing them under hypoxic conditions (less than 20% oxygen), such as for example about 2% or about 0% oxygen.

Akt1 is an intracellular serine/threonine protein kinase that plays a key role in multiple cellular processes such as glucose metabolism, cell proliferation, apoptosis, transcription and cell migration. Overexpression of Akt1 has been shown to prevent rat MSCs from undergoing apoptosis and will have the same effect in the IMP cells of the invention. Protection from apoptosis will enhance the therapeutic effect of the IMP cells.

The overexpression of HGF by MSCs has been shown to prevent post-ischemic heart failure by inhibition of apoptosis via calcineurin-mediated pathway and angiogenesis. HGF and G-CSF exhibit synergistic effects in this regard. MSCs that have a high expression of HGF and its receptor c-met also have an increased migratory capacity into the damaged tissue, achieved through hormonal, paracrine and autocrine signaling. The same will be true for the IMP cells of the invention expressing HGF and/or G-CSF.

The IMP cells may express detectable levels off one or more of (i) to (vii) defined above. The IMP cells of the invention preferably express an increased amount of one or more of (i) to (vii) compared with MSCs. Quantitative assays for cell markers are described above. The detectable expression of these markers and their level of expression may be measured as discussed above.

Any of the IMP cells may express detectable levels of one or more of (i) vascular endothelial growth factor (VEGF), (ii) transforming growth factor beta (TGF-beta), (iii) insulin-like growth factor-1 (IGF-1), (iv) fibroblast growth factor (FGF), (v) tumour necrosis factor alpha (TNF-alpha), (vi) interferon gamma (IFN-gamma) and (vii) interleukin-1 alpha (IL-1 alpha). Conditioned medium from cells overexpressing VEGF has been shown to alleviate heart failure in a hamster model. Hence, the IMP cells of the invention which express or express an increased amount of VEGF will have the same effect of damaged cardiac tissue.

The IMP cells may express detectable levels of one or more of (i) to (vii). The IMP cells may express an increased amount of one or more of (i) to (vii) compared with MSCs. Quantitative assays for cell markers are described above. The detectable expression of these markers and their level of expression may be measured as discussed above.

5 In both sets of definitions of (i) to (vii) given above, any combination of one or more of (i) to (vii) may be expressed or expressed in an increased amount. For instance, for each definition of (i) to (vii), the IMP cells may express detectable levels of, or express an increased amount of, (i); (ii); (iii); (iv); (v); (vi); (vii); (i) and (ii); (i) and (iii); (i) and (iv); (i) and (v); (i) and (vi); (i) and (vii); (ii) and (iii); (ii) and (iv); (ii) and (v); (ii) and (vi); (ii) and (vii); (iii) and (iv); (iii) and (v); (iii) and (vi); (iii) and (vii); (iv) and (v); (iv) and (vi); (iv) and (vii); (v) and (vi); (v) and (vii); (vi) and (vii); (i), (ii) and (iii); (i), (ii) and (iv); (i), (ii) and (v); (i), (ii) and (vi); (i), (ii) and (vii); (i), (iii) and (iv); (i), (iii) and (v); (i), (iii) and (vi); (i), (iii) and (vii); (i), (iv) and (v); (i), (iv) and (vi); (i), (iv) and (vii); (i), (v) and (vi); (i), (v) and (vii); (i), (vi) and (vii); (ii), (iii) and (iv); (ii), (iii) and (v); (ii), (iii) and (vi); (ii), (iii) and (vii); (ii), (iv) and (v); (ii), (iv) and (vi); (ii), (iv) and (vii); (ii), (v) and (vi); (ii), (v) and (vii); (ii), (vi) and (vii); (iii), (iv) and (v); (iii), (iv) and (vi); (iii), (iv) and (vii); (iii), (v) and (vi); (iii), (v) and (vii); (iii), (vi) and (vii); (iv), (v) and (vi); (iv), (v) and (vii); (iv), (vi) and (vii); (v), (vi) and (vii); (i), (ii), (iii) and (iv); (i), (ii), (iii) and (v); (i), (ii), (iii) and (vi); (i), (ii), (iii) and (vii); (i), (ii), (iv) and (v); (i), (ii), (iv) and (vi); (i), (ii), (iv) and (vii); (i), (ii), (v) and (vi); (i), (ii), (v) and (vii); (i), (ii), (vi) and (vii); (i), (iii), (iv) and (v); (i), (iii), (iv) and (vi); (i), (iii), (iv) and (vii); (i), (iii), (v) and (vi); (i), (iii), (v) and (vii); (i), (iii), (vi) and (vii); (i), (iv), (v) and (vi); (i), (iv), (v) and (vii); (i), (iv), (vi) and (vii); (i), (v), (vi) and (vii); (ii), (iii), (iv) and (v); (ii), (iii), (iv) and (vi); (ii), (iii), (iv) and (vii); (ii), (iii), (v) and (vi); (ii), (iii), (v) and (vii); (ii), (iii), (vi) and (vii); (ii), (iv), (v) and (vi); (ii), (iv), (v) and (vii); (ii), (iv), (vi) and (vii); (ii), (v), (vi) and (vii); (iii), (iv), (v) and (vi); (iii), (iv), (v) and (vii); (iii), (iv), (vi) and (vii); (iii), (v), (vi) and (vii); (iv), (v), (vi) and (vii); (i), (ii), (iii), (iv) and (v); (i), (ii), (iii), (iv) and (vi); (i), (ii), (iii), (iv) and (vii); (i), (ii), (iii), (v) and (vi); (i), (ii), (iii), (v) and (vii); (i), (ii), (iii), (vi) and (vii); (i), (ii), (iv), (v) and (vi); (i), (ii), (iv), (v) and (vii); (i), (ii), (iv), (vi) and (vii); (i), (ii), (v), (vi) and (vii); (i), (iii), (iv), (v) and (vi); (i), (iii), (iv), (v) and (vii); (i), (iii), (iv), (vi) and (vii); (i), (iii), (v), (vi) and (vii); (i), (iv), (v), (vi) and (vii); (ii), (iii), (iv), (v) and (vi); (ii), (iii), (iv), (v) and (vii); (ii), (iii), (iv), (v), (vi) and (vii); (ii), (iii), (v), (vi) and (vii); (ii), (iv), (v), (vi) and (vii); (iii), (iv), (v), (vi) and (vii); (i), (ii), (iii), (iv), (v) and (vi); (i), (ii), (iii), (iv), (v) and (vii); (i), (ii), (iii), (iv), (vi) and (vii); (i), (ii), (iii), (v), (vi) and (vii); (i), (ii), (iv), (v), (vi) and (vii); (i), (iii), (iv), (v), (vi) and (vii); (ii), (iii), (iv), (v), (vi) and (vii); (ii), (iii), (iv), (v), (vi) and (vii); (ii), (iv), (v), (vi) and (vii); (iii), (iv), (v), (vi) and (vii); (i), (ii), (iii), (iv), (v) and (vi); (i), (ii), (iii), (iv), (v) and (vii); (i), (ii), (iii), (iv), (vi) and (vii); (i), (ii), (iii), (v), (vi) and (vii); (i), (ii), (iv), (v), (vi) and (vii); (i), (iii), (iv), (v), (vi) and (vii); (ii), (iii), (iv), (v), (vi) and (vii); or (i), (ii), (iii), (iv), (v), (vi) and (vii). The combinations for each definition of (i) to (vii) are independently selectable from this list.

In addition to any of the markers discussed above, the IMP cells preferably also express detectable levels of, LIF and/or platelet-derived growth factor (PDGF) receptors. The IMP cells of the invention preferably express an increased amount of LIF and/or platelet-derived growth factor (PDGF) receptors compared with mesenchymal stem cells. The PDGF receptors are preferably PDGF-A
5 receptors and/or PSDGF-B receptors. MSCs that have high expression of these receptors can migrate effectively into areas in which platelets have been activated, such as wounds and thrombotic vessels. The same will be true of IMP cells expressing or expressing an increased amount of the receptors.

The PMLs and/or the IMP cells are preferably autologous. In other words, the cells are preferably derived from the patient into which the cells will be administered. Alternatively, the PMLs
10 and/or IMP cells are preferably allogeneic. In other words, the cells are preferably derived from a patient that is immunologically compatible with the patient into which the cells will be administered.

PMLs and/or IMP cells may be isolated using a variety of techniques including antibody-based techniques. Cells may be isolated using negative and positive selection techniques based on the binding of monoclonal antibodies to those surface markers which are present on the PMLs and/or IMP
15 cells (see above). Hence, the PMLs and/or IMP cells may be separated using any antibody-based technique, including fluorescent activated cell sorting (FACS) and magnetic bead separation.

As discussed in more detail below, the PMLs and/or IMP cells may be treated *ex vivo*. Thus the cells may be loaded or transfected with a therapeutic or diagnostic agent and then used therapeutically in the methods of the invention.

The one or more PMLs and/or IMP cells may be produced using any known method. The one
20 or more PMLs are preferably produced using the method described in International Application No. PCT/GB2012/051600 (published as WO 2013/005053). The one or more IMP cells are preferably produced using the method disclosed in the UK Application being filed concurrently with this application (CTL Ref. IMP1).

This typically involves culturing mononuclear cells (MCs) under conditions which induce the MCs to differentiate into PMLs or IMP cells and then harvesting and culturing the PMLs or IMP cells which express the markers discussed above.

Mononuclear cells (MCs) and methods of isolating them are known in the art. The MCs may be primary MCs isolated from bone marrow. The MCs are preferably peripheral blood MCs (PBMCs),
30 such as lymphocytes, monocytes and/or macrophages. PBMCs can be isolated from blood using a hydrophilic polysaccharide, such as Ficoll®. For instance, PBMCs may be isolated from blood using Ficoll-Paque® (a commercially-available density medium) as disclosed in the Example.

Before they are cultured, the MCs may be exposed to a mesenchymal stem cell enrichment cocktail. The cocktail preferably comprises antibodies that recognise CD3, CD14, CD19, CD38, CD66b (which are present on unwanted cells) and a component of red blood cells. Such a cocktail cross links unwanted cells with red blood cells forming immunorosettes which may be removed from the wanted MCs. A preferred cocktail is RosetteSep®.

Conditions suitable for inducing MCs to differentiate into mesenchymal cells (tissue mainly derived from the mesoderm) are known in the art. For instance, suitable conditions are disclosed in Capelli, C., *et al.* (Human platelet lysate allows expansion and clinical grade production of mesenchymal stromal cells from small samples of bone marrow aspirates or marrow filter washouts. Bone Marrow Transplantation, 2007. 40: p. 785-791). These conditions may also be used to induce MCs to differentiate into PMLs in accordance with the invention.

The method preferably comprises culturing MCs with plasma lysate to induce the MCs to differentiate into PMLs or IMP cells. Platelet lysate refers to the combination of natural growth factors contained in platelets that has been released through lysing those platelets. Lysis can be accomplished through chemical means (i.e. CaCl_2), osmotic means (use of distilled H_2O) or through freezing/thawing procedures. Platelet lysate can be derived from whole blood as described in U.S. Pat. No. 5,198,357. Platelet lysate is preferably prepared as described in PCT/GB12/052911 (published as WO 2013/076507). The plasma lysate is preferably human plasma lysate.

For instance, the MCs may be cultured in a medium comprising platelet lysate for sufficient time to induce the MCs to differentiate into PMLs or IMP cells. The sufficient time is typically from about 15 to about 25 days, preferably about 22 days. The medium preferably comprises about 20% or less platelet lysate by volume, such as about 15% or less by volume or about 10% or less by volume. The medium preferably comprises from about 5% to about 20% of platelet lysate by volume, such as from about 10% to about 15% by volume. The medium preferably comprises about 10% of platelet lysate by volume.

Alternatively, the MCs may be exposed to a mesenchymal enrichment cocktail and then cultured in a medium comprising platelet lysate for sufficient time to induce the MCs to differentiate into PMLs or IMP cells. The sufficient time is typically from about 15 to about 25 days, preferably about 22 days.

The medium is preferably Minimum Essential Medium (MEM). MEM is commercially available from various sources including Sigma-Aldrich. The medium preferably further comprises one or more of heparin, L-glutamine and penicillin/streptavidin (P/S). The L-glutamine may be replaced with GlutaMAX® (which is commercially-available from Life Technologies).

The MCs are typically cultured under conditions which allow the PMLs or IMP cells to adhere. Suitable conditions are discussed in more detail above.

The MCs are preferably cultured under low oxygen conditions. The MCs are preferably cultured at less than about 20% oxygen (O_2), such as less than about 19%, less than about 18%, less than about 17%, less than about 16%, less than about 15%, less than about 14%, less than about 13%, less than about 12%, less than about 11%, less than about 10%, less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2% or less than about 1% oxygen (O_2). The MCs are preferably cultured at from about 0% to about 19% O_2 , such as from about 1% to about 15% O_2 , from about 2% to about 10% O_2 or from about 5% to about 8% O_2 . The MCs are most preferably cultured at about 0% O_2 . The figures for % oxygen (or % O_2) quoted above relate to % by volume of oxygen in the gas supplied to the cells during culture, for instance by the cell incubator. It is possible that some oxygen may leak into the incubator or enter when the door is opened.

The MCs are most preferably cultured in the presence of platelet lysate and under low oxygen conditions. This combination mimics the natural conditions in the damaged tissue and so result in healthier and more therapeutically potent cells. Conventional cell culture is performed in 20% or 21% oxygen (approximately the atmospheric content) but there is no place in the human body that has this oxygen level. The epithelial cells in the lungs would “see” this oxygen level, but once the oxygen is dissolved and leaves the lungs, it decreases to around 17%. From there, it decreases even further to about 1-2% in the majority of the tissues, but being as low as 0.1% in avascular tissues such as the cartilage in the joints.

Producing one or more PMLs or IMP cells also comprises harvesting and culturing PMLs or IMP cells which have the necessary marker expression pattern as discussed above. The PMLs or IMP cells having the necessary marker expression pattern may be harvested using any antibody-based technique, including fluorescent activated cell sorting (FACS) and magnetic bead separation. FACS is preferred.

As will be clear from the discussion above, the production of one or more PMLs or IMP cells is carried out in clinically relevant conditions, i.e. in the absence of trace amounts of endotoxins and other environmental contaminants, such as lipopolysaccharides, lipopeptides and peptidoglycans, etc. This makes the PMLs or IMP cells particularly suitable for administration to patients.

The MCs are preferably obtained from a patient or an allogeneic donor.

The one or more therapeutic cells are preferably one or more mesenchymal stem cells (MSCs). Suitable MSCs are known in the art. Any of the MSCs disclosed above may be used.

The one or more therapeutic cells are preferably one or more mesenchymal precursor cells (MPCs). The one or more therapeutic cells are more preferably one or more human mesenchymal precursor cells (MPCs).

The one or more therapeutic cells are preferably one or more dendritic cells.

5 The one or more therapeutic cells are preferably one or more platelets.

The one or more therapeutic cells are preferably one or more fibroblasts.

The one or more therapeutic cells are preferably one or more myofibroblasts.

10 The one or more therapeutic cells are preferably human. The one or more therapeutic cells may be derived from any of the animals or mammals discussed above with reference to the source of the PML and IMP cells.

15 The one or more therapeutic cells are typically cultured *in vitro* before being combined with the other components in the composition. Techniques for culturing cells are well known to a person skilled in the art. The cells are may be cultured under standard conditions of 37°C, 5% CO₂ in medium without serum. The cells are preferably cultured under low oxygen conditions as discussed in more detail below. The cells may be cultured in any suitable flask or vessel, including wells of a flat plate such as a standard 6 well plate. Such plates are commercially available from Fisher scientific, VWR suppliers, Nunc, Starstedt or Falcon. The wells typically have a capacity of from about 1ml to about 4ml.

20 The flask, vessel or wells within which the population is contained or cultured may be modified to facilitate handling of the cells. For instance, the flask, vessel or wells may be modified to facilitate culture of the cells, for instance by including a growth matrix. The flask, vessel or wells may be modified to allow attachment of the cells or to allow immobilization of the cells onto a surface. One or more surfaces may be coated with extracellular matrix proteins such as laminin or collagen or any other capture molecules that bind to the cells and immobilize or capture them on the surface(s).

25 The cells may be modified *ex vivo* using any of the techniques described herein. For instance, the population may be transfected or loaded with therapeutic or diagnostics agents. The population may then be used in the methods of treatment discussed in more detail below.

Preferred compositions

30 Preferred hybrid compositions of the invention are summarised in the Table below.

	Fibre(s)	Cell(s)	Component(s)	Format
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1	PLGA	MSC, MPC, IMP, PML or mixture thereof	Fibrin (gel)	Cell(s) attached to the fibre(s)
2	PLGA	MSC, MPC, IMP, PML or mixture thereof	Integrin	Cell(s) attached to the fibre(s)
3	PLGA	MSC, MPC, IMP, PML or mixture thereof	Cadherin	Cell(s) attached to the fibre(s)
4	PLGA	MSC, MPC, IMP, PML or mixture thereof	Fibrin (gel)	Cell(s) embedded in the component(s)
5	PLGA	MSC, MPC, IMP, PML or mixture thereof	Integrin	Cell(s) embedded in the component(s)
6	PLGA	MSC, MPC, IMP, PML or mixture thereof	Cadherin	Cell(s) embedded in the component(s)

Liposome composition

The invention also provides a liposome composition comprising one or more hybrid compositions of the invention contained within one or more liposomes. Suitable liposomes are known in the art. Suitable liposomes are disclosed in, for example, Akbarzadeh et al. Nanoscale Research Letters 2013, 8:102 and Meghana et al. International Journal Of Pharmaceutical And Chemical Sciences, 2012, 1(1): 1-10. Suitable lipids for use in forming liposomes are discussed below with reference to microbubbles.

Microbubble composition

The invention also provides a microbubble composition comprising one or more hybrid compositions of the invention contained within one or more microbubbles. Microbubbles, their

formation and biomedical uses are known in the art (*e.g.* Sirsi and Borden, Bubble Sci Eng Technol. Nov 2009; 1(1-2): 3–17).

Microbubbles are bubbles smaller than one millimetre in diameter and larger than one micrometre in diameter. The microbubble of the present invention is preferably 8µm or less in diameter, such as 7µm or less in diameter, 6µm or less in diameter, 5µm or less in diameter, 4µm or less in diameter, 3µm or less in diameter or 2µm or less in diameter.

The microbubble may be formed from any substance. The general composition of a microbubble is a gas core stabilised by a shell. The gas core may comprise air or a heavy gas, such as perfluorocarbon, nitrogen or perfluoropropane. Heavy gases are less water soluble and so are less likely to leak out from the microbubble leading to microbubble dissolution. Microbubbles with heavy gas cores typically last longer in circulation.

The shell may be formed from any material. The shell material preferably comprises a protein, a surfactant, a lipid, a polymer or a mixture thereof.

Suitable proteins, include but are not limited to, albumin, lysozyme and avidin. Proteins within the shell may be chemically-crosslinked, for instance by cysteine-cysteine linkage. Other crosslinkages are known in the art.

Suitable surfactants include, but are not limited to, sorbitan monopalmitate (such as SPAN-40), polysorbate detergents (such as TWEEN-40), mixtures of SPAN-40 and TWEEN-40 and sucrose stearate (mono- and di-ester).

Suitable polymers include, but are not limited to, alginate polymers, double ester polymers of ethylidene, the copolymer poly(D,L-lactide-co-glycolide) (PLGA), poly(vinyl alcohol) (PVA), the copolymer polyperfluorooctyloxycaronyl-poly(lactic acid) (PLA-PFO) and other block copolymers. Block copolymers are polymeric materials in which two or more monomer sub-units that are polymerized together to create a single polymer chain. Block copolymers typically have properties that are contributed by each monomer sub-unit. However, a block copolymer may have unique properties that polymers formed from the individual sub-units do not possess. Block copolymers can be engineered such that one of the monomer sub-units is hydrophobic (*i.e.* lipophilic), whilst the other sub-unit(s) are hydrophilic whilst in aqueous media. In this case, the block copolymer may possess amphiphilic properties and may form a structure that mimics a biological membrane. The block copolymer may be a diblock (consisting of two monomer sub-units), but may also be constructed from more than two monomer sub-units to form more complex arrangements that behave as amphiphiles. The copolymer may be a triblock, tetrablock or pentablock copolymer. Block copolymers may also be constructed from sub-units that are not classed as lipid sub-materials; for example a hydrophobic

polymer may be made from siloxane or other non-hydrocarbon based monomers. The hydrophilic sub-section of block copolymer can also possess low protein binding properties, which allows the creation of a membrane that is highly resistant when exposed to raw biological samples. This head group unit may also be derived from non-classical lipid head-groups.

Any lipid material that forms a microbubble may be used. The lipid composition is chosen such that the microbubble has the required properties, such surface charge, packing density or mechanical properties. The lipid composition can comprise one or more different lipids. For instance, the lipid composition can contain up to 100 lipids. The lipid composition preferably contains 1 to 10 lipids. The lipid composition may comprise naturally-occurring lipids and/or artificial lipids.

The lipid typically comprises a head group, an interfacial moiety and two hydrophobic tail groups which may be the same or different. Suitable head groups include, but are not limited to, neutral head groups, such as diacylglycerides (DG) and ceramides (CM); zwitterionic head groups, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE) and sphingomyelin (SM); negatively charged head groups, such as phosphatidylglycerol (PG); phosphatidylserine (PS), phosphatidylinositol (PI), phosphatic acid (PA) and cardiolipin (CA); and positively charged headgroups, such as trimethylammonium-Propane (TAP). Suitable interfacial moieties include, but are not limited to, naturally-occurring interfacial moieties, such as glycerol-based or ceramide-based moieties. Suitable hydrophobic tail groups include, but are not limited to, saturated hydrocarbon chains, such as lauric acid (*n*-Dodecanolic acid), myristic acid (*n*-Tetradecanoic acid), palmitic acid (*n*-Hexadecanoic acid), stearic acid (*n*-Octadecanoic) and arachidic (*n*-Eicosanoic); unsaturated hydrocarbon chains, such as oleic acid (*cis*-9-Octadecanoic); and branched hydrocarbon chains, such as phytanoyl. The length of the chain and the position and number of the double bonds in the unsaturated hydrocarbon chains can vary. The length of the chains and the position and number of the branches, such as methyl groups, in the branched hydrocarbon chains can vary. The hydrophobic tail groups can be linked to the interfacial moiety as an ether or an ester.

The lipids can also be chemically-modified. The head group or the tail group of the lipids may be chemically-modified. Suitable lipids whose head groups have been chemically-modified include, but are not limited to, PEG-modified lipids, such as 1,2-Diacyl-sn-Glycero-3-Phosphoethanolamine-N - [Methoxy(Polyethylene glycol)-2000]; functionalised PEG Lipids, such as 1,2-Distearoyl-sn-Glycero-3 Phosphoethanolamine-N-[Biotinyl(Polyethylene Glycol)2000]; and lipids modified for conjugation, such as 1,2-Dioleoyl-sn-Glycero-3-Phosphoethanolamine-N-(succinyl) and 1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-(Biotinyl). Suitable lipids whose tail groups have been chemically-modified include, but are not limited to, polymerisable lipids, such as 1,2-bis(10,12-tricosadiynoyl)-sn-

Glycero-3-Phosphocholine; fluorinated lipids, such as 1-Palmitoyl-2-(16-Fluoropalmitoyl)-sn-Glycero-3-Phosphocholine; deuterated lipids, such as 1,2-Dipalmitoyl-D62-sn-Glycero-3-Phosphocholine; and ether linked lipids, such as 1,2-Di-O-phytanyl-sn-Glycero-3-Phosphocholine. The lipids may be chemically-modified or functionalised to facilitate coupling of the ligands, receptors or antibodies as discussed above.

The lipid composition may comprise one or more additives that will affect the properties of the microbubble. Suitable additives include, but are not limited to, fatty acids, such as palmitic acid, myristic acid and oleic acid; fatty alcohols, such as palmitic alcohol, myristic alcohol and oleic alcohol; sterols, such as cholesterol, ergosterol, lanosterol, sitosterol and stigmasterol; lysophospholipids, such as 1-Acyl-2-Hydroxy-sn- Glycero-3-Phosphocholine; and ceramides.

The microbubble shell is preferably formed from a phospholipid. Suitable phospholipids are known in the art.

There are several commercially available lipid shell microbubble formulations such as Definity (Lantheus Medical Imaging) and Sonovue® (Bracco Diagnostics).

The microbubble may also be formed from a polymer-surfactant hybrid that involves forming polyelectrolyte multilayer (PEM) shells on a preformed microbubble. The preformed microbubble is coated with a charged surfactant or protein layer, which serves as a substrate for PEM deposition. The layer-by-layer assembly technique is used to sequentially adsorb oppositely charged polyions to the microbubble shell. For instance, PEM can be deposited onto microbubbles using poly(allylamine hydrochloride) (PAH) and poly(styrene sulfonate) (PSS) for the polyion pair. PEM microbubbles with phospholipid containing the cationic headgroup trimethylammonium propane (TAP) as the underlying shell and DNA and poly(L-lysine) (PLL) as the polyion pair have also been developed.

The microbubble is typically formed by providing an interface between a gas and a microbubble shell material. Any of the materials discussed above may be used. Some materials, such as phospholipids, spontaneously form microbubbles. Phospholipids self assemble into a microbubble. Other materials require sonication of the interface, i.e. the application of sound energy or sonic waves to the interface. Ultrasonic waves are typically used. Suitable methods are known in the art for sonication.

The microbubble may be loaded with the hybrid composition after formation of the microbubble or during formation of the microbubble.

Medicaments, methods and therapeutic use

The hybrid composition of the invention or the liposome or microbubble composition of the invention may be used in a method of therapy of the human or animal body. Thus the invention provides a hybrid composition of the invention or a liposome or microbubble composition of the invention for use in a method of treatment of the human or animal body by therapy. In particular, the invention concerns using the hybrid composition of the invention or the liposome or microbubble composition of the invention to repair a damaged tissue in a patient. The invention also concerns using a hybrid composition of the invention or a liposome or microbubble composition of the invention to treat a cardiac, bone, cartilage, tendon, ligament, liver, kidney or lung injury or disease in the patient.

The invention provides a method of repairing a damaged tissue in a patient, comprising contacting the damaged tissue with a hybrid composition of the invention or a liposome or microbubble composition of the invention, wherein the composition comprises a therapeutically effective number of cells, and thereby treating the damaged tissue in the patient. The invention also provides a hybrid composition of the invention or a liposome or microbubble composition of the invention for use in repairing a damaged tissue in the patient. The invention also provides use of a hybrid composition of the invention or a liposome or microbubble composition of the invention in the manufacture of a medicament for repairing a damaged tissue in a patient.

The tissue is preferably derived from the mesoderm. The tissue is more preferably cardiac, bone, cartilage, tendon, ligament, liver, kidney or lung tissue.

The damage to the tissue may be caused by injury or disease. The injury or disease is preferably a cardiac, bone, cartilage, tendon, ligament, liver, kidney or lung injury or disease in a patient. The invention therefore provides a method of treating a cardiac, bone, cartilage, tendon, ligament, liver, kidney or lung injury or disease in a patient, comprising administering to the patient a hybrid composition of the invention or a liposome or microbubble composition of the invention, wherein the composition comprises a therapeutically effective number of cells, and thereby treating the injury or disease in the patient. The invention also provides a hybrid composition of the invention or a liposome or microbubble composition of the invention for use in treating a cardiac, bone, cartilage, tendon, ligament, liver, kidney or lung injury or disease in a patient. The invention also provides use of a hybrid composition of the invention or a liposome or microbubble composition of the invention in the manufacture of a medicament for treating a cardiac, bone, cartilage, tendon, ligament, liver, kidney or lung injury or disease in a patient.

The cardiac injury or disease is preferably selected from myocardial infarct (MI), left ventricular hypertrophy, right ventricular hypertrophy, emboli, heart failure, congenital heart deficit, heart valve disease, arrhythmia and myocarditis.

MI increases the levels of VEGF and EPO released by the myocardium. Furthermore, MI is associated with an inflammatory reaction and infarcted tissue also releases macrophage migration inhibitory factor (MIF), interleukin (IL-6) and KC/Gro-alpha. CCL7 (previously known as MCP3), CXCL1, CXCL2 are significantly upregulated in the heart following myocardial infarct (MI) and might be implicated in regulating engraftment and homing of MSCs to infarcted myocardium.

In a myocardial infarct mice model, IL-8 was shown to highly up-regulate gene expression primarily in the first 2 days post-MI. Remarkably, the increased IL-8 expression was located predominantly in the infarcted area and the border zone, and only to a far lesser degree in the spared myocardium. By activating CXCR2, MIF displays chemokine-like functions and acts as a major regulator of inflammatory cell recruitment and atherogenesis.

The bone disease or injury is preferably selected from fracture, Salter-Harris fracture, greenstick fracture, bone spur, craniosynostosis, Coffin-Lowry syndrome, fibrodysplasia ossificans progressive, fibrous dysplasia, Fong Disease (or Nail-patella syndrome), hypophosphatasia, Klippel-Feil syndrome, Metabolic Bone Disease, Nail-patella syndrome, osteoarthritis, osteitis deformans (or Paget's disease of bone), osteitis fibrosa cystica (or Osteitis fibrosa or Von Recklinghausen's disease of bone), osteitis pubis, condensing osteitis (or osteitis condensans), osteitis condensans ilii, osteochondritis dissecans, osteogenesis imperfecta, osteomalacia, osteomyelitis, osteopenia, osteopetrosis, osteoporosis, osteonecrosis, porotic hyperostosis, primary hyperparathyroidism, renal osteodystrophy, bone cancer, a bone lesion associated with metastatic cancer, Gorham Stout disease, primary hyperparathyroidism, periodontal disease, and aseptic loosening of joint replacements. The bone cancer can be Ewing sarcoma, multiple myeloma, osteosarcoma (giant tumour of the bone), osteochondroma or osteoclastoma. The metastatic cancer that results in a bone lesion can be breast cancer, prostate cancer, kidney cancer, lung cancer and/or adult T-cell leukemia.

In all instances, the one or more therapeutic cells are preferably derived from the patient or an allogeneic donor. Deriving the cells from the patient should ensure that the cells are themselves not rejected by the patient's immune system. Any difference between the donor and recipient will ultimately cause clearance of the cells, but not before they have repaired at least a part of the damaged tissue.

The invention concerns administering to the patient a therapeutically effective number of therapeutic cells to the patient. A therapeutically effective number is a number which ameliorates one or more symptoms of the damage, disease or injury. A therapeutically effective number is preferably a number which repairs the damaged tissue or treats the disease or injury. Suitable numbers are discussed in more detail below.

The hybrid composition of the invention or liposome or microbubble composition of the invention may be administered to any suitable patient. The patient is generally a human patient. The patient may be any of the animals or mammals mentioned above with reference to the source of the PML and IMP cells.

5 The patient may be an infant, a juvenile or an adult. The patient may be known to have a damaged tissue or is suspected of having a damaged tissue. The patient may be susceptible to, or at risk from, the relevant disease or injury. For instance, the patient may be genetically predisposed to heart failure.

10 The invention may be used in combination with other means of, and substances for, repairing damaged tissue or providing pain relief. In some cases, the hybrid composition of the invention or a liposome or microbubble composition of the invention may be administered simultaneously, sequentially or separately with other substances which are intended for repairing the damaged tissue or for providing pain relief. The hybrid composition of the invention or a liposome or microbubble composition of the invention may be used in combination with existing treatments for damaged tissue
15 and may, for example, be simply mixed with such treatments. Thus the invention may be used to increase the efficacy of existing treatments of damaged tissue.

20 The invention preferably concerns the use of therapeutic cells loaded or transfected with a therapeutic and/or diagnostic agent. A therapeutic agent may help to repair the damaged tissue. A diagnostic agent, such as a fluorescent molecule, may help to identify the location of the composition in the patient. The cells may be loaded or transfected using any method known in the art. The loading of cells may be performed *in vitro* or *ex vivo*. In each case, the cells may simply be in contact with the agent in culture. Alternatively, the cells may be loaded with an agent using delivery vehicle, such as liposomes. Such vehicles are known in the art.

25 The transfection of cells, such as PMLs or IMP cells, may be performed *in vitro* or *ex vivo*. Alternatively, stable transfection may be performed at the MC stage allowing PMLs expressing the transgene to be differentiated from them. The cells are typically transfected with a nucleic acid encoding the agent. For instance, viral particles or other vectors encoding the agent may be employed. Methods for doing this are known in the art.

30 The nucleic acid gives rise to expression of the agent in the cells. The nucleic acid molecule will preferably comprise a promoter which is operably linked to the sequences encoding the agent and which is active in the PMLs or which can be induced in the cells.

 In a particularly preferred embodiment, the nucleic acid encoding the agent may be delivered via a viral particle. The viral particle may comprise a targeting molecule to ensure efficient

transfection. The targeting molecule will typically be provided wholly or partly on the surface of the virus in order for the molecule to be able to target the virus to the cells.

Any suitable virus may be used in such embodiments. The virus may, for example, be a retrovirus, a lentivirus, an adenovirus, an adeno-associated virus, a vaccinia virus or a herpes simplex virus. In a particularly preferred embodiment the virus may be a lentivirus. The lentivirus may be a modified HIV virus suitable for use in delivering genes. The lentivirus may be a SIV, FIV, or equine infectious anemia virus (EQIA) based vector. The virus may be a moloney murine leukaemia virus (MMLV). The viruses used in the invention are preferably replication deficient.

Viral particles do not have to be used. Any vector capable of transfecting the cells may be used, such as conventional plasmid DNA or RNA transfection.

Uptake of nucleic acid constructs may be enhanced by several known transfection techniques, for example those including the use of transfection agents. Examples of these agents includes cationic agents, for example, calcium phosphate and DEAE-Dextran and lipofectants, for example, lipofectAmine, fugene and transfectam.

The cells may be loaded or transfected under suitable conditions. The cells and agent or vector may, for example, be contacted for between five minutes and ten days, preferably from an hour to five days, more preferably from five hours to two days and even more preferably from twelve hours to one day.

In some embodiments, MCs may be recovered from a patient, converted into PMLs and/or IMP cells as discussed above, loaded or transfected *in vitro* and then returned to the same patient. In such instances, the PMLs and/or IMP cells employed in the invention, will be autologous cells and fully matched with the patient. In a preferred case, the cells employed in the invention are recovered from a patient and utilised *ex vivo* and subsequently returned to the same patient.

Pharmaceutical compositions and administration

The composition of the invention may be formulated using any suitable method. Formulation of cells with standard pharmaceutically acceptable carriers and/or excipients may be carried out using routine methods in the pharmaceutical art. The exact nature of a formulation will depend upon several factors including the cells to be administered and the desired route of administration. Suitable types of formulation are fully described in Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing Company, Eastern Pennsylvania, USA.

The composition may be administered by any route. Suitable routes include, but are not limited to, intravenous, intramuscular, intraperitoneal or other appropriate administration routes. The composition is preferably administered directly to the damaged tissue.

Compositions may be prepared together with a physiologically acceptable carrier or diluent.

- 5 The composition may be mixed with an excipient which is pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, of the like and combinations thereof.

In addition, if desired, the pharmaceutical compositions of the invention may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or
10 adjuvants which enhance effectiveness. The composition preferably comprises human serum albumin.

One suitable carrier or diluents is Plasma-Lyte A®. This is a sterile, nonpyrogenic isotonic solution for intravenous administration. Each 100 mL contains 526 mg of Sodium Chloride, USP (NaCl); 502 mg of Sodium Gluconate (C₆H₁₁NaO₇); 368 mg of Sodium Acetate Trihydrate, USP (C₂H₃NaO₂•3H₂O); 37 mg of Potassium Chloride, USP (KCl); and 30 mg of Magnesium Chloride,
15 USP (MgCl₂•6H₂O). It contains no antimicrobial agents. The pH is adjusted with sodium hydroxide. The pH is 7.4 (6.5 to 8.0).

The composition is administered in a manner compatible with the dosage formulation and in such amount will be therapeutically effective. The quantity of the therapeutic cells to be administered depends on the subject to be treated, capacity of the subject's immune system and the degree repair
20 desired. Precise amounts of cells required to be administered may depend on the judgement of the practitioner and may be peculiar to each subject.

Any suitable number of cells may be administered to a subject. For example, at least, or about, 0.2×10^6 , 0.25×10^6 , 0.5×10^6 , 1.5×10^6 , 4.0×10^6 or 5.0×10^6 cells per kg of patient may administered. For example, at least, or about, 10^5 , 10^6 , 10^7 , 10^8 , 10^9 cells may be administered. As a
25 guide, the number of cells to be administered may be from 10^5 to 10^9 , preferably from 10^6 to 10^8 . Typically, up to 2×10^8 IMP cells are administered to each patient. Any of the specific numbers discussed above may be administered.

The following Example illustrates the invention.

30 **Example**

Hybrid compositions were manufactured according to the schemes shown in Figures 3 to 6.

CLAIMS

1. A hybrid composition, which comprises:
 - (a) one or more biocompatible fibres;
 - (b) one or more therapeutic cells; and
 - (c) one or more biocompatible components which (i) attach the one or more therapeutic cells to the one or more fibres and/or embed the one or more therapeutic cells and the one or more fibres or (ii) are capable of attaching the composition to a tissue.
2. A hybrid composition according to claim 1, wherein the one or more fibres are one or more cellulose fibres, one or more collagen fibres, one or more collagen-glycosaminoglycan fibres, one or more gelatin fibres, one or more silk fibroin fibres, one or more fibrin fibres, one or more chitosan fibres, one or more starch fibres, one or more alginate fibres, one or more hyaluronan fibres, one or more poloxamer fibres or a combination thereof.
3. A hybrid composition according to claim 2, wherein the glycosaminoglycan is chondroitin.
4. A hybrid composition according to any one of the preceding claims, wherein the one or more biocompatible components comprise a biocompatible adhesive which attaches the one or more therapeutic cells to the one or more fibres.
5. A hybrid composition according to claim 4, wherein the biocompatible adhesive attaches the one or more therapeutic cells on the surface of the one or more fibres and/or within the one or more fibres.
6. A hybrid composition according to claim 4 or 5, wherein the biocompatible adhesive is fibrin gel, integrin or cadherin.
7. A hybrid composition according to any one of claims 4 to 6, wherein the biocompatible adhesive comprises platelet lysate.

8. A hybrid composition according to any one of the preceding claims, wherein the one or more biocompatible components comprise a biocompatible gel which embeds the one or more therapeutic cells and the one or more fibres.
9. A hybrid composition according to claim 8, wherein the biocompatible gel is a cellulose gel, a collagen gel, a gelatin gel, a fibrin gel, a chitosan gel, a starch gel, an alginate gel, a hyaluronan gel, an agarose gel, a poloxamer gel or a combination thereof.
10. A hybrid composition according to claim 8 or 9, wherein the biocompatible gel comprises platelet lysate.
11. A hybrid composition according to any one of the preceding claims, wherein the one or more therapeutic cells comprise one or more progenitor cells of mesodermal lineage (PMLs), one or more immuno-modulatory progenitor (IMP) cells, one or more mesenchymal stem cells, one or more dendritic cells, one or more platelets, one or more fibroblasts, one or more myofibroblasts or a combination thereof.
12. A hybrid composition according to claim 11, wherein the one or more PMLs (a) express detectable levels of CD29, CD44, CD73, CD90, CD105 and CD271 and (b) do not express detectable levels of CD14, CD34 and CD45.
13. A hybrid composition according to claim 12, wherein the one or more PMLs express detectable levels of CD62P (P-selectin) and /or CD62E (E-selectin).
14. A hybrid composition according to claim 12, wherein the one or more IMP cells express detectable levels of MIC A/B, CD304 (Neuropilin 1), CD178 (FAS ligand), CD289 (Toll-like receptor 9), CD363 (Sphingosine-1-phosphate receptor 1), CD99, CD181 (C-X-C chemokine receptor type 1; CXCR1), epidermal growth factor receptor (EGF-R), CXCR2 and CD126.
15. A hybrid composition according to claim 12 or 14, the one or more IMP cells express detectable levels of one or more of CD10, CD111, CD267, CD47, CD273, CD51/CD61, CD49f, CD49d, CD146, CD55, CD340, CD91, Notch2, CD175s, CD82, CD49b, CD95, CD63, CD245, CD58,

CD108, B2-microglobulin, CD155, CD298, CD44, CD49c, CD105, CD166, CD230, HLA-ABC, CD13, CD29, CD49e, CD59, CD73, CD81, CD90, CD98, CD147, CD151 and CD276.

16. A hybrid composition according to any one of claims 12, 14 or 15, wherein the one or more IMP cells express:

(a) detectable levels of one or more of CD156b, CD61, CD202b, CD130, CD148, CD288, CD337, SSEA-4, CD349, CD140b, CD10, CD111, CD267, CD47, CD273, CD51/CD61, CD49f, CD49d, CD146, CD55, CD340, CD91, Notch2, CD175s, CD82, CD49b, CD95, CD63, CD245, CD58, CD108, B2-microglobulin, CD155, CD298, CD44, CD49c, CD105, CD166, CD230, HLA-ABC, CD13, CD29, CD49e, CD59, CD73, CD81, CD90, CD98, CD147, CD151 and CD276; and/or

(b) detectable levels of one or more of CD72, CD133, CD192, CD207, CD144, CD41b, FMC7, CD75, CD3e, CD37, CD158a, CD172b, CD282, CD100, CD94, CD39, CD66b, CD158b, CD40, CD35, CD15, PAC-1, CLIP, CD48, CD278, CD5, CD103, CD209, CD3, CD197, HLA-DM, CD20, CD74, CD87, CD129, CDw329, CD57, CD163, TPBG, CD206, CD243 (BD), CD19, CD8, CD52, CD184, CD107b, CD138, CD7, CD50, HLA-DR, CD158e2, CD64, DCIR, CD45, CLA, CD38, CD45RB, CD34, CD101, CD2, CD41a, CD69, CD136, CD62P, TCR alpha beta, CD16b, CD1a, ITGB7, CD154, CD70, CDw218a, CD137, CD43, CD27, CD62L, CD30, CD36, CD150, CD66, CD212, CD177, CD142, CD167, CD352, CD42a, CD336, CD244, CD23, CD45RO, CD229, CD200, CD22, CDH6, CD28, CD18, CD21, CD335, CD131, CD32, CD157, CD165, CD107a, CD1b, CD332, CD180, CD65 and CD24.

17. A hybrid composition according to any one of the preceding claims, wherein the one or more therapeutic cells are autologous or allogeneic.

18. A liposome composition comprising one or more hybrid compositions according to any one of the preceding claims contained within one or more liposomes.

19. A microbubble composition comprising one or more hybrid compositions according to any one of claims 1 to 17 contained within one or more microbubbles.

20. A method of producing a hybrid composition according to any one of claims 1 to 17, comprising (a) providing one or more biocompatible fibres, one or more therapeutic cells and one or more biocompatible components and (b) attaching the one or more therapeutic cells to the one or more

fibres using one or more biocompatible components and/or embedding the one or more therapeutic cells and the one or more fibres in one or more biocompatible components or combining the one or more fibres, the one or more cells and the one or more components and thereby producing a hybrid composition according to any one of claims 1 to 17.

21. A method of repairing a damaged tissue in a patient, comprising contacting the damaged tissue with a hybrid composition according to any one of claims 1 to 17, a liposome composition according to claim 18 or a microbubble composition according to claim 19, wherein the composition comprises a therapeutically effective number of cells, and thereby treating the damaged tissue in the patient.

22. A method according to claim 21, wherein the comprises implanting or injecting the hybrid composition or the liposome composition into the damaged tissue or adhering or anchoring the hybrid composition or the liposome composition to the damaged tissue.

23. A method according to claim 21 or 22, wherein the one or more fibres are approximately the same length as the depth of damage in the tissue.

24. A method according to any one of claims 21 to 23, wherein the tissue is derived from the mesoderm.

25. A method according to claim 24, wherein the tissue is cardiac, bone, cartilage, tendon, ligament, liver, kidney or lung tissue.

26. A method according to any one of claims 21 to 25, wherein the tissue is damaged by injury or disease.

27. A method according to claim 26, wherein the method is for treating a cardiac, bone, cartilage, tendon, ligament, liver, kidney or lung injury or disease in the patient.

28. A method according to claim 27, wherein the cardiac injury or disease is selected from myocardial infarct, left ventricular hypertrophy, right ventricular hypertrophy, emboli, heart failure, congenital heart deficit, heart valve disease, arrhythmia and myocarditis.

29. A method according to claim 25, wherein the bone injury or disease is selected from fracture, Salter-Harris fracture, greenstick fracture, bone spur, craniosynostosis, Coffin-Lowry syndrome, fibrodysplasia ossificans progressive, fibrous dysplasia, Fong Disease (or Nail-patella syndrome), hypophosphatasia, Klippel-Feil syndrome, Metabolic Bone Disease, Nail-patella syndrome, osteoarthritis, osteitis deformans (or Paget's disease of bone), osteitis fibrosa cystica (or Osteitis fibrosa or Von Recklinghausen's disease of bone), osteitis pubis, condensing osteitis (or osteitis condensans), osteitis condensans ilii, osteochondritis dissecans, osteogenesis imperfecta, osteomalacia, osteomyelitis, osteopenia, osteopetrosis, osteoporosis, osteonecrosis, porotic hyperostosis, primary hyperparathyroidism, renal osteodystrophy, bone cancer, a bone lesion associated with metastatic cancer, Gorham Stout disease, primary hyperparathyroidism, periodontal disease, and aseptic loosening of joint replacements.
30. A method according to any one of claims 21 to 29, wherein the population of therapeutic cells is obtained from the patient or an allogeneic donor.
31. A hybrid composition according to any one of claims 1 to 17, a liposome composition according to claim 18 or a microbubble composition according to claim 19 for use in a method of repairing a damaged tissue in a patient.
32. A hybrid composition according to any one of claims 1 to 17, a liposome composition according to claim 18 or a microbubble composition according to claim 19 for use in a method of treating a cardiac, bone, cartilage, tendon, ligament, liver, kidney or lung injury or disease in a patient.