METHODS FOR DIFFERENTIATING MESENCHYMAL STEM CELLS

FIELD OF THE INVENTION

The invention relates to methods for expansion and/or differentiation of mesenchymal stem cells (MSC), to MSC-derived cells and cell populations, and to products comprising such cells and cell populations, methods and uses.

BACKGROUND

Transplantation of stem cells capable of undergoing osteogenic differentiation, of cells that are committed towards osteogenic differentiation or of cells with bone-forming ability is a promising avenue for the treatment of bone-related diseases, in particular when the treatment requires production of new bone tissues.

Mesenchymal stem cells (MSC) have been used previously to treat bone disorders (Gangji et al., 2005 Expert Opin Biol Ther 5: 437-42). However, although such relatively undifferentiated stem cells can be transplanted, they are not committed to an osteoblastic lineage and therefore a considerable proportion of so transplanted stem cells may not eventually contribute to the formation of the desired bone tissue. Moreover, the quantity of such stem cells is frequently unsatisfactory.

WO 2007/093431 concerns a method for *in vitro* expansion of isolated MSC, which yielded cells displaying an osteoblastic phenotype. In said method, human MSC were cultured in the presence of serum or plasma and basic fibroblast growth factor (FGF-2).

WO 2009/087213 concerns a method for obtaining osteoprogenitors, osteoblasts or osteoblast phenotype cells from human MSC *in vitro* or *ex vivo*, comprising contacting said MSC with human plasma or serum, FGF-2 and transforming growth factor beta (TGF-β).

There exists a continuous need for novel cells and cell populations useful in among others therapy, such as novel MSC-derived cells and cell populations, and methods for producing the same.

SUMMARY

In one aspect, the present disclosure refers to a method for obtaining mesenchymal stem cell (MSC)-derived cells from MSC comprising contacting MSC *in vitro* or *ex vivo* with fibroblast growth factor-2 (FGF-2), transforming growth factor beta (TGFβ) and heparin or a derivative or analogue thereof at a concentration of at least 0.01 IU/ml.

In another aspect, the present disclosure refers to a method for obtaining MSC-derived cells with improved transplantation properties from MSC, the method comprising a size reduction step, wherein said size reduction step is characterized by contacting MSC or MSC-derived cells

in vitro or *ex vivo* with heparin or a derivative or analogue thereof at a concentration of at least 0.01 IU/ml.

In yet another aspect, the present disclosure refers to a method for obtaining MSC-derived cells from MSC comprising contacting MSC *in vitro* or *ex vivo* with FGF-2, TGFβ and heparin or a derivative or analogue thereof, whereby at least 60% of the MSC-derived cells in suspension have a diameter equal to or less than 25 µm ($D_{60} \leq 25$ µm) and wherein at most 5% of the MSC-derived cells in suspension have a diameter of more than 35 µm.

In a further aspect, the present disclosure refers to a population of MSC-derived cells, which are cells of mesenchymal lineage obtained by *in vitro* or *ex vivo* differentiation of MSC, whereby at least 60% of the MSC-derived cells in suspension have a diameter equal to or less than 25 µm ($D_{60} \leq 25$ µm) and wherein at most 5 % of the MSC-derived cells in suspension have a diameter of more than 35 µm.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising the population of MSC-derived cells disclosed herein.

In yet another aspect, the present disclosure refers to the population of MSC-derived cells disclosed herein, or the pharmaceutical composition disclosed herein, for use as a medicament, preferably for use in the treatment of a subject in need of transplantation of MSC-derived cells.

As corroborated by the experimental section, which illustrates certain representative examples of the present disclosure, the inventors realized that the transplantation potential of mesenchymal stem cells (MSC)-derived cells can be considerably augmented when said cells are obtained by contacting MSC or MSC-derived cells *in vitro* or *ex vivo* with heparin or a derivative or analogue. More particularly, the inventors found that contacting MSC or MSC-derived cells with heparin or a derivative or analogue, preferably heparin or a derivative or analogue thereof at a concentration of at least 0.01 IU/ml, gives rise to new MSC-cell derived cell populations of which the cells have a standardized, homogeneous, and comparatively small

cell size. Such MSC-cell derived cells have improved transplantation properties, such as (i) improved suitability for parenteral (e.g., intravascular, including intravenous) administration, (ii) the possibility to deliver *in vivo* a tunable and high cell concentration with a limited volume, (iii) a good *in vivo* safety profile and/or (iv) a good syringeability when delivered parenterally.

Furthermore, the present MSC-derived cells are capable to induce bone formation. In addition to the osteo-inductive properties, the present inventors found that the present MSC-derived cells also display a high osteogenic activity. The osteogenic activity advantageously leads to the occurrence of mineralized nodules produced through endochondral ossification.

Hence, in an example, the present disclosure provides a method for obtaining MSC-derived cells with improved transplantation properties from MSC, the method comprising a size reduction step, wherein said size reduction step is characterized by contacting MSC or MSC-derived cells *in vitro* or *ex vivo* with heparin or a derivative or analogue thereof at a concentration of at least 0.01 IU/ml.

In a further example the present disclosure provides a method for obtaining MSC-derived cells from MSC comprising contacting MSC *in vitro* or *ex vivo* with FGF-2, TGFβ and heparin or a derivative or analogue thereof at a concentration of at least 0.01 IU/ml.

In a further example, the present disclosure provides a method for obtaining MSC-derived cells from MSC comprising contacting MSC *in vitro* or *ex vivo* with FGF-2, TGFβ and heparin or a derivative or analogue thereof, whereby at least 60% of the MSC-derived cells in suspension have a diameter equal to or less than 25 µm ($D_{60} \leq 25$ µm) and wherein at most 5% of the MSC-derived cells in suspension have a diameter of more than 35 µM.

In another example, the present disclosure provides a population of MSC-derived cells obtainable by *in vitro* or *ex vivo* expansion of MSC, whereby at least 60% of the MSC-derived cells in suspension have a diameter equal to or less than 25 µm ($D_{60} \leq 25$ µm) and wherein at most 5% of the MSC-derived cells in suspension have a diameter of more than 35 µM.

In another example, the present disclosure provides a pharmaceutical composition comprising the population of MSC-derived cells as taught herein.

In another example, the present disclosure provides the population of MSC-derived cells or the pharmaceutical composition as taught herein for use as a medicament.

These and further examples and preferred examples of the present disclosure are described in the following sections and in the appended claims. The subject-matter of the appended claims is hereby specifically incorporated in this specification.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the cell size of MSC-derived cells, particularly MSC-derived bone-forming cells, generated with fibroblast growth factor-2 (FGF-2) and transforming growth factor beta 1

CLAIMS

1. A method for obtaining mesenchymal stem cell (MSC)-derived cells from MSC comprising contacting MSC *in vitro* or *ex vivo* with fibroblast growth factor-2 (FGF-2), transforming growth factor beta (TGFβ) and heparin or a derivative or analogue thereof at a concentration of at least 0.01 IU/ml.

2. The method according to claim 1, comprising the steps of:

(a) culturing MSC recovered from a biological sample of a subject in a medium comprising FGF-2, TGFβ and heparin or derivative or analogue thereof at a concentration of at least 0.01 IU/ml;

(b) removing non-adherent matter and further culturing adherent cells in the medium comprising FGF-2, TGFβ and heparin or a derivative or analogue thereof at a concentration of at least 0.01 IU/ml, thereby obtaining the MSC-derived cells.

3. The method according to claim 1 or 2, wherein TGFβ is selected from the group consisting of TGFβ1, TGFβ2, TGFβ3, and mixtures thereof; preferably wherein TGFβ is TGFβ1.

4. A method for obtaining MSC-derived cells with improved transplantation properties from MSC, the method comprising a size reduction step, wherein said size reduction step is characterized by contacting MSC or MSC-derived cells *in vitro* or *ex vivo* with heparin or a derivative or analogue thereof at a concentration of at least 0.01 IU/ml.

5. The method according to any one of claims 1 to 4, wherein:

- the concentration of heparin or derivative or analogue thereof is at least 0.05 IU/ml, preferably about 0.1 IU/ml; and/or

- heparin or heparin derivative or analogue thereof is selected from the group consisting of unfractionated heparin (UFH); low molecular weight heparin (LMWH), such as enoxaparin, dalteparin, nadroparin, tinzaparin, certoparin, reviparin, ardeparin, parnaparin, bemiparin, or mixtures thereof; a heparinoid, such as heparan sulfate, dermatan sulfate, chondroitin sulfate, acharan sulfate, keratan sulfate, or mixtures thereof, such as danaparoid; a heparin salt; a heparinoid salt; a heparin fragment; a heparinoid fragment; and mixtures thereof.

6. The method according to any one of claims 1 to 5, whereby at least 60% of the MSC-derived cells in suspension have a diameter equal to or less than 25 µm ($D_{60} \leq 25$ µm) and wherein at most 5% of the MSC-derived cells in suspension have a diameter of more than 35 µm.

7. The method according to any one of claims 1 to 6, wherein the MSC-derived cells are of osteochondroblastic, preferably of osteoblastic lineage.

8. The method according to any one of claims 1 to 7, wherein the MSC or MSC-derived cells are additionally contacted with, such as wherein the medium additionally comprises one or more of, plasma, serum or a substitute thereof.

9. A method for obtaining MSC-derived cells from MSC comprising contacting MSC *in vitro* or *ex vivo* with FGF-2, TGFβ and heparin or a derivative or analogue thereof, whereby at least 60% of the MSC-derived cells in suspension have a diameter equal to or less than 25 µm ($D_{60} \leq 25$ µm) and wherein at most 5% of the MSC-derived cells in suspension have a diameter of more than 35 µm.

10. The method according to claim 9, wherein:

- MSC are contacted with heparin or derivative or analogue thereof at a concentration of at least 0.01 IU/ml; and/or

- TGFβ is selected from the group consisting of TGFβ1, TGFβ2, TGFβ3, and mixtures thereof; preferably wherein TGFβ is TGFβ1.

11. A population of MSC-derived cells, which are cells of mesenchymal lineage obtained by *in vitro* or *ex vivo* differentiation of MSC, whereby at least 60% of the MSC-derived cells in suspension have a diameter equal to or less than 25 µm ($D_{60} \leq 25$ µm) and wherein at most 5 % of the MSC-derived cells in suspension have a diameter of more than 35 µm.

12. The population of MSC-derived cells according to claim 11, wherein:

- the MSC-derived cells are obtainable by a method comprising contacting MSC *in vitro* or *ex vivo* with FGF-2, TGFβ and heparin or a derivative or analogue thereof, preferably wherein TGFβ is selected from the group consisting of TGFβ1, TGFβ2, TGFβ3, and mixtures thereof, more preferably wherein TGFβ is TGFβ1, preferably wherein MSC are contacted with heparin or derivative or analogue thereof at a concentration of at least 0.01 IU/ml, and optionally the MSC are additionally contacted with one or more of plasma, serum or a substitute thereof; and/or

- the MSC-derived cells are of osteochondroblastic or osteoblastic lineage, preferably wherein substantially all MSC-derived cells of osteochondroblastic or osteoblastic lineage are positive for CD73, CD63 and CD166; substantially all MSC-derived cells of osteochondroblastic or osteoblastic lineage are negative for CD45; at least 70% of the MSC-derived cells of osteochondroblastic lineage or osteoblastic are positive for alkaline phosphatase (ALP); and less than 10% of the MSC-derived cells of osteochondroblastic or osteoblastic lineage are positive for HLA-DR.

13. A pharmaceutical composition comprising the population of MSC-derived cells as defined in claim 11 or 12.

14. The population of MSC-derived cells according to claim 11 or 12 or the pharmaceutical composition according to claim 13 for use as a medicament, preferably for use in the treatment of a subject in need of transplantation of MSC-derived cells.

15. The population of MSC-derived cells for use according to claim 14, wherein:

- the population of MSC-derived cells is present at a concentration between about $1x10^7$/ml and about $1x10^8$/ml, preferably 7.5×10^7 cells/ml; and/or

- the population of MSC-derived cells or the pharmaceutical composition is suitable for percutaneous or intravascular administration.