

SUMMARY

The present disclosure provides an *in vitro* method for assessing, evaluating and/or monitoring the purity of a cell preparation comprising mesenchymal stem cells (MSC), wherein said method comprises measuring the expression level of at least one growth factor expressed by said cell preparation, wherein said at least one growth factor is SDF-1 α and/or VEGF.

In one aspect, the present disclosure refers to an *in vitro* method for assessing, evaluating and/or monitoring the purity of a heterogeneous cell preparation comprising mesenchymal stem cells (MSC) with respect to fibroblastic contamination, and wherein said method comprises the steps of (i) culturing said cell preparation comprising MSCs, (ii) measuring the expression level of at least one growth factor expressed by said cell preparation, wherein said at least one growth factor is SDF-1 α and/or VEGF and (iii) comparing the measured expression level with a reference expression level.

According to one embodiment, the method of the present disclosure further comprises comparing the measured expression level with a reference expression level.

In one embodiment, the mesenchymal stem cells of the cell preparation of the present disclosure are isolated from tissues selected from the group comprising adipose tissue, bone marrow, umbilical cord blood, amniotic fluid, Wharton's jelly, placenta, peripheral blood, fallopian tube, corneal stroma, lung, muscle and fetal liver. In a particular embodiment, the mesenchymal stem cells are adipose stem cells (ASC).

According to one embodiment, the expression level of at least one growth factor expressed by said cell preparation of the present disclosure is assessed at the protein level, preferably by the detection and/or quantification of said at least one growth factor secreted in the cell culture supernatant. In a particular embodiment, the expression level is assessed at the RNA level, preferably by RT-PCR, RT-qPCR, Northern Blot and/or hybridization techniques.

According to one embodiment, the cell preparation of the present disclosure is substantially pure when the SDF-1 α expression level is of at most 100 pg/ml, preferably of at most 50, 40, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 pg/ml.

According to another embodiment, the cell preparation of the present disclosure is substantially pure when the SDF-1 α expression level is of at most 100 pg/ml, preferably of at most 50, 40, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 pg/ml; and/or the VEGF expression level is of at least 200 pg/ml in the cell culture medium, preferably of at least 250, 260, 270, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289 or 290 pg/ml; wherein said cell preparation is cultured in hypoxic conditions, preferably at about 0.1%

O₂, and at high concentration of glucose, preferably at about 4.5 g/l of glucose, before measuring the expression level.

According to another embodiment, the cell preparation of the present disclosure is substantially pure when the SDF-1 α expression level is of at most 100 pg/ml, preferably of at most 50, 40, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 pg/ml; and/or the VEGF expression level is of at least 90 pg/ml in the cell culture medium, preferably of at least 95, 100, 105, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 or 120 pg/ml, wherein said cell preparation is cultured at tissular oxygen tension, preferably at about 5% O₂, and at high concentration of glucose, preferably at about 4.5 g/l of glucose, before measuring the expression level.

In one embodiment, the method of the present disclosure is for assessing the quality or purity of a cell preparation comprising mesenchymal stem cells (MSC), wherein said cell preparation comprising MSC is to be used as MSC-based cell therapy product in regenerative medicine.

The present disclosure also provides a use of a growth factor, preferably SDF-1 α and/or VEGF, as a biomarker of the quality of a cell preparation comprising mesenchymal stem cells (MSC), in particular of a cell preparation comprising MSC to be used as MSC-based cell therapy product in regenerative medicine.

In another aspect of the present disclosure, there is provided a use of SDF-1 α and/or VEGF as a biomarker of the quality of a cell preparation comprising mesenchymal stem cells (MSC), in particular of a cell preparation comprising MSC to be used as MSC-based cell therapy product in regenerative medicine.

The present disclosure also provides a cell population identified by the *in vitro* method as described hereinabove.

The present disclosure also relates to a substantially pure mesenchymal stem cells population, preferably an adipose stem cells population.

The present disclosure also provides a kit for implementing the *in vitro* method as described hereinabove, wherein said kit comprises means for determining or measuring the expression level of at least one growth factor, optionally further comprising the reference for comparing the expression level of said at least one growth factor.

In another aspect of the present disclosure, there is provided a kit for implementing the *in vitro* method as described hereinabove, wherein said kit comprises means for determining or measuring the expression level of SDF-1 α and/or VEGF, and wherein said kit comprises a pure fibroblasts preparation and/or a pure mesenchymal stem cell preparation as reference for comparing the expression level of said SDF-1 α and/or VEGF.

CLAIMS

1. An *in vitro* method for assessing, evaluating and/or monitoring the purity of a heterogeneous cell preparation comprising mesenchymal stem cells (MSC) with respect to fibroblastic contamination, and wherein said method comprises the steps of (i) culturing said cell preparation comprising MSCs, (ii) measuring the expression level of at least one growth factor expressed by said cell preparation, wherein said at least one growth factor is SDF-1 α and/or VEGF and (iii) comparing the measured expression level with a reference expression level.
2. The *in vitro* method according to claim 1, wherein said mesenchymal stem cells are isolated from tissues selected from the group comprising adipose tissue, bone marrow, umbilical cord blood, amniotic fluid, Wharton's jelly, placenta, peripheral blood, fallopian tube, corneal stroma, lung, muscle and fetal liver.
3. The *in vitro* method according to claim 1 or 2, wherein said mesenchymal stem cells are adipose stem cells (ASC).
4. The *in vitro* method according to any one of claims 1 to 3, wherein said expression level is assessed at the protein level, preferably by the detection and/or quantification of said at least one growth factor secreted in the cell culture supernatant.
5. The *in vitro* method according to any one of claims 1 to 3, wherein said expression level is assessed at the protein level or at the RNA level, preferably by RT-PCR, RT-qPCR, Northern Blot and/or hybridization techniques.
6. The *in vitro* method according to any one of claims 1 to 5, wherein said cell preparation is substantially pure when the SDF-1 α expression level is of at most 100 pg/ml, preferably of at most 50, 40, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 pg/ml.
7. The *in vitro* method according to any one of claims 1 to 6, wherein said cell preparation is substantially pure when the SDF-1 α expression level is of at most 100 pg/ml, preferably of at most 50, 40, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11,

10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 pg/ml; and/or the VEGF expression level is of at least 200 pg/ml in the cell culture medium, preferably of at least 250, 260, 270, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289 or 290 pg/ml; wherein said cell preparation is cultured in hypoxic conditions, preferably at about 0.1% O₂, and at high concentration of glucose, preferably at about 4.5 g/l of glucose, before measuring the expression level.

8. The *in vitro* method according to any one of claims 1 to 6, wherein said cell preparation is substantially pure when the SDF-1 α expression level is of at most 100 pg/ml, preferably of at most 50, 40, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 pg/ml; and/or the VEGF expression level is of at least 90 pg/ml in the cell culture medium, preferably of at least 95, 100, 105, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119 or 120 pg/ml, wherein said cell preparation is cultured at tissular oxygen tension, preferably at about 5% O₂, and at high concentration of glucose, preferably at about 4.5 g/l of glucose, before measuring the expression level.
9. Use of SDF-1 α and/or VEGF as a biomarker of the quality of a cell preparation comprising mesenchymal stem cells (MSC), in particular of a cell preparation comprising MSC to be used as MSC-based cell therapy product in regenerative medicine.

- 10.** A kit for implementing the *in vitro* method according to any one of claims **1** to **8**, wherein said kit comprises means for determining or measuring the expression level of SDF-1 α and/or VEGF, and wherein said kit comprises a pure fibroblasts preparation and/or a pure mesenchymal stem cell preparation as reference for comparing the expression level of said SDF-1 α and/or VEGF.