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(71) Applicants: **CELLRESEARCH CORPORATION PTE. LTD.** [SG/SG]; 137 Market Street, #08-02, Grace Global Raffles Building, Singapore 048943 (SG). **THE REGEN-**

TS OF THE UNIVERSITY OF COLORADO [US/US]; 1800 Grant Street, 8th Floor, Denver, Colorado 80203 (US).

(72) Inventors: **PHAN, Toan Thang**; 137 Market Street, #08-02, Grace Global Raffles Building, Singapore 048943 (SG). **FREED, Brian M.**; Division of Allergy and Clinical Immunology, University of Colorado Anschutz Medical Campus, 12705 E. Montview Blvd. Ste 250, Aurora, Colorado 80045 (US).

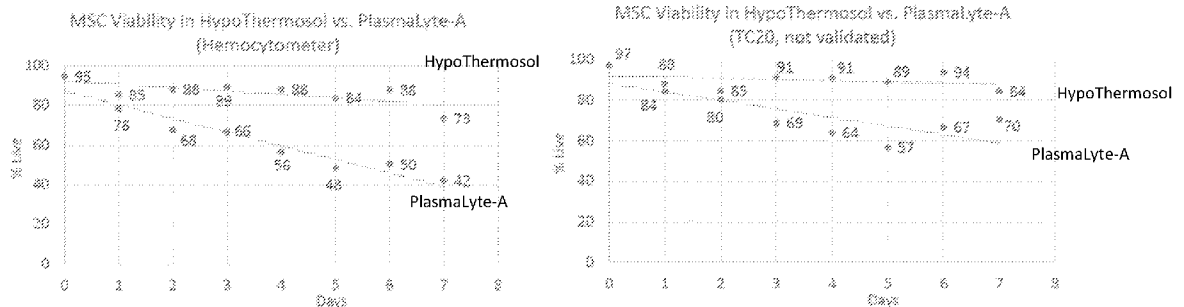
(74) Agent: **MCLAUGHLIN, Michael Gerard** et al.; McLaughlin IP PTE. LTD., #14-01, 112 Robinson Road, Singapore 068902 (SG).

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(54) Title: A METHOD OF TRANSPORTING MESENCHYMAL STEM CELLS BY MEANS OF A TRANSPORTING SOLUTION AND A METHOD OF ADMINISTERING STEM CELLS TO WOUNDS

Figure 9

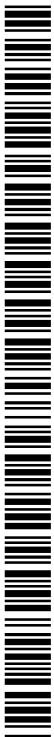
Exp. # 180105



| total cell counts | Hemocytometer | Std. dev. | % std. dev. | TC20 | Std. dev. | % std. dev. |
|-------------------|---------------|-----------|-------------|-----------|-----------|-------------|
| HypoThermosol | 802,500 | 118,155 | 14.8 | 758,337 | 104,984 | 13.8 |
| PlasmaLyte | 1,608,871 | 213,902 | 13.3 | 1,301,571 | 208,981 | 16.1 |

- HypoThermosol noticeably supports membrane integrity and presumably survival over a week timespan (6 days).
- All counts based on duplicate readings that are within 10% of one another. Following SOP CR D2.600.1.
- During counting, cells stored in HypoThermosol were noticeably smaller with smooth and defined edges. By contrast, cells in PlasmaLyte appeared of a range of sizes.

(57) Abstract: The present invention relates to a method of transporting a stem cell population, the method comprising transporting the stem cell population contacted with a liquid carrier. In the preferred embodiment, the liquid carrier is HypoThermosol and comprises Trolox e.g. HypoThermosol-FRS. In addition, the present invention concerns a method of treating a subject having a disease such as a wound, the method comprising topically administering a defined mesenchymal stem cell population to the subject, wherein the mesenchymal stem cell population is administered within about 96 hours from the time point the mesenchymal stem cell population has been harvested. Also concerned is a unit dosage comprising the defined mesenchymal stem cell population such as from the amniotic membrane of the umbilical cord, wherein at least 90% of the cells express each of CD73, CD90 and CD105.



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**A METHOD OF TRANSPORTING MESENCHYMAL STEM CELLS BY MEANS
OF A TRANSPORTING SOLUTION AND A METHOD OF ADMINISTERING
STEM CELLS TO WOUNDS**

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] The present application claims the benefit of priority of U.S. Provisional Application No. 62/655,198, filed April 9, 2018, the content of which is hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[002] The present invention relates to a method of transporting a stem cell population, the method comprising transporting the stem cell population contacted with a liquid carrier. In addition, the present invention concerns a method of treating a subject having a disease, the method comprising topically administering a defined mesenchymal stem cell population to the subject, wherein the mesenchymal stem cell population is administered within about 96 hours from the time point the mesenchymal stem cell population has been harvested. Also concerned is a unit dosage comprising about 20 million cells, of about 15 million cells, of about 10 million cells, of about 5 million cells, of about 4 million cells, of about 3 million cells, of about 2 million cells, of about 1 million cells, of about 0.5 million cells, of about 0.25 million cells or of less than 0.25 million cells of a defined mesenchymal stem cell population.

BACKGROUND OF THE INVENTION

[003] Mesenchymal stem cells isolated from the amniotic membrane of the umbilical cord and their wound healing properties have been first reported in US patent application 2006/0078993 (leading to granted US patents 9,085,755, 9,737,568 and 9,844,571) and the corresponding International patent application WO2006/019357. Since then, the umbilical cord tissue has gained attention as a source of multipotent cells; due to its widespread availability, the umbilical cord and in particular stem cells isolated from the amniotic membrane of the umbilical cord (also referred to as “cord lining stem cells”) have been

considered as an excellent alternative source of cells for regenerative medicine. See, Jeschke et al. Umbilical Cord Lining Membrane and Wharton's Jelly-Derived Mesenchymal Stem Cells: the Similarities and Differences; *The Open Tissue Engineering and Regenerative Medicine Journal*, 2011, 4, 21-27.

[004] A subsequent study compared the phenotype, proliferation rate, migration, immunogenicity, and immunomodulatory capabilities of human mesenchymal stem cells (MSCs) derived from the amniotic membrane of the umbilical cord (umbilical cord lining (CL-MSCs), umbilical cord blood (CB-MSCs), placenta (P-MSCs), and Wharton's jelly (WJ-MSCs) (Stubbendorf et al, *Immunological Properties of Extraembryonic Human Mesenchymal Stromal Cells Derived from Gestational Tissue, STEM CELLS AND DEVELOPMENT* Volume 22, Number 19, 2013, 2619-2629. Stubbendorf et al concluded that extraembryonic gestational tissue-derived MSC populations show a varied potential to evade immune responses as well as exert immunomodulatory effects. The authors also found that CL-MSCs showed the most promising potential for a cell-based therapy, as the cells showed low immunogenicity, but they also showed enhanced proliferative and migratory potential so that future research should concentrate on the best disease models in which CL-MSCs could be administered.

[005] While mesenchymal stem cells of the amniotic membrane can easily be obtained using the protocol as described in US patent application 2006/0078993 and International patent application WO2006/019357, it would be of advantage for clinical trials with these cord lining MSC to have at hand a method that allows to isolate a population of these cord lining MSC's that is highly homogenous and can thus be used for clinical trials.

[006] Such a highly homogenous population of mesenchymal stem cells derived from the amniotic membrane of the umbilical cord has been reported for the first time in co-pending US application Serial No. 15/725,913, filed 5 October 2018 claiming priority to US provisional application Serial No. 62/404,582 filed 5 October 2017, the content of both of which is incorporated by reference herein in its entirety) and as well as in co-pending PCT application PCT/SG2017/050500 also filed 5 October 2018 claiming priority to US provisional application No. 62/404,582 filed 5 October 2017 and meets the criteria for mesenchymal stem cells to be used for cellular therapy (also cf. the Experimental Section of US application Serial No. 15/725,913, Dominici et al, "Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement", *Cytotherapy* (2006) Vol. 8, No. 4, 315-317, or Sensebe et

al., "Production of mesenchymal stromal/stem cells according to good manufacturing practices: a review", Stem Cell Research & Therapy 2013, 4:66.

[007] Stem cells such as the mesenchymal stem cells as described above are however typically not applied/administered to patients at the site where they are produced. Often a substantial amount of time passes in between the harvesting of cells and their further utilization. There is thus a need for the provision of certain carriers which keep cells viable and healthy for a period of time typically used for transport or storage of cells.

[008] Accordingly, it is an object of the invention to provide a method of transporting/storing stem cells, especially of a population of mesenchymal stem cells from the amniotic membrane of umbilical cord that meets this need.

SUMMARY OF THE INVENTION

[009] This object is accomplished by the methods and the unit dosage having the features of the independent claims.

[0010] In a first aspect, the invention provides a method of transporting a stem cell population, the method comprising transporting said stem cell population contacted with a liquid carrier, said liquid carrier comprising

- i) Trolox;
- ii) Na⁺;
- iii) K⁺;
- iv) Cl⁻;
- v) H₂PO₄⁻;
- vi) HEPES;
- vii) Lactobionate;
- viii) Sucrose;
- ix) Mannitol;
- x) Glucose;
- xi) Dextran-40;
- xii) Adenosine, and
- xiii) Glutathione.

[0011] In a second aspect, the invention provides a method of treating a subject having a disease, the method comprising topically administering a mesenchymal stem cell population as described herein to the subject, wherein the mesenchymal stem cell population

is administered within about 96 hours from the time point the mesenchymal stem cell population has been harvested.

[0012] In a third aspect, the invention provides a unit dosage comprising about 20 million cells, of about 15 million cells, of about 10 million cells, of about 5 million cells, of about 4 million cells, of about 3 million cells, of about 2 million cells, of about 1 million cells, of about 0.5 million cells, of about 0.25 million cells or of less than 0.25 million cells of a mesenchymal stem cell population as described herein.

[0013] In a fourth aspect, the invention provides the use of a liquid carrier for transporting a stem cell population, wherein the liquid carrier comprises

- i) Trolox;
- ii) Na+;
- iii) K+;
- iv) Cl-;
- v) H₂PO₄-;
- vi) HEPES;
- vii) Lactobionate;
- viii) Sucrose;
- ix) Mannitol;
- x) Glucose;
- xi) Dextran-40;
- xii) Adenosine, and
- xiii) Glutathione.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the drawings, in which:

[0015] **Fig. 1** shows the technical information sheet of Lonza for Dulbecco's modified eagle medium, including the catalogue number of the DMEM used for the making of the illustrative example of a medium of the invention (PTT-6) in the Experimental Section;

[0016] **Fig. 2** shows the technical information sheet of Lonza for Ham's F12 medium;

[0017] **Fig. 3** shows the technical information sheet of Lonza for DMEM:F12 (1:1) medium, including the catalogue number of the DMEM:F12 (1:1) medium used for the

making of the illustrative example of a medium of the invention (PTT-6) in the Experimental Section;

[0018] **Fig. 4** shows the technical information sheet of Life Technologies Corporation for M171 medium, including the catalogue number of the M171 medium used for the making of the illustrative example of a medium of the invention (PTT-6) in the Experimental Section;

[0019] **Fig. 5** shows the list of ingredients, including their commercial supplier and the catalogue number that have been used in the Experimental Section for the making of the medium PTT-6.

[0020] **Fig. 6** shows the results of flow cytometry experiments in which mesenchymal stem cells isolated from the umbilical cord have been analysed for the expression of the mesenchymal stem cell markers CD73, CD90 and CD105. For these experiments, mesenchymal stem cells were isolated from umbilical cord tissue by cultivation of the umbilical cord tissue in three different cultivation media, followed by subculturing of the mesenchymal stem cells in the respective medium. The three following culture media were used in these experiments: a) 90% (v/v) DMEM supplemented with 10 % FBS (v/v), b) the culture medium PTT-4 described in US patent application 2006/0078993 and the corresponding International patent application WO2006/019357 that consist of 90% (v/v) CMRL1066, and 10% (v/v) FBS (see paragraph [0183] of WO2006/019357 and c) the culture medium of the present invention PTT-6 the composition of which is described herein. In this flow cytometry analysis, two different samples of the cord lining mesenchymal stem cell (CLMC) population were analysed for each of the three used culture media. The results are shown in **Fig. 6a** to **Fig.6c**. In more detail, **Fig. 6a** shows the percentage of isolated mesenchymal cord lining stem cells expressing stem cell markers CD73, CD90 and CD105 after isolation from umbilical cord tissue and cultivation in DMEM/10% FBS, **Fig. 6b** shows the percentage of isolated mesenchymal cord lining stem cells expressing stem cell markers CD73, CD90 and CD105 after isolation from umbilical cord tissue and cultivation in PTT-4 and **Fig. 6c** shows the percentage of isolated mesenchymal cord lining stem cells expressing stem cell markers CD73, CD90 and CD105 after isolation from umbilical cord tissue and cultivation in PTT-6.

[0021] **Fig. 7** shows the results of flow cytometry experiments in which mesenchymal stem cells isolated from the umbilical cord have been analysed for their expression of stem cells markers (CD73, CD90 and CD105, CD34, CD45 and HLA-DR (Human Leukocyte

Antigen – antigen D Related) that are used for defining the suitability of multipotent human mesenchymal stem cells for cellular therapy and compared to the expression of these markers by bone marrow mesenchymal stem cells. For this experiment, the mesenchymal stem cells of the amniotic membrane of the umbilical cord were isolated from umbilical cord tissue by cultivation of the umbilical cord tissue in the culture medium of the present invention PTT-6 while the bone marrow mesenchymal stem cells were isolated from human bone marrow using a standard protocol.

[0022] Fig. 7a shows the percentage of isolated mesenchymal cord lining stem cells that express the stem cell markers CD73, CD90 and CD105 and lack expression of CD34, CD45 and HLA-DR after isolation from umbilical cord tissue and cultivation in PTT-6 medium while Fig. 7b shows the percentage of isolated bone marrow mesenchymal stem cells that express CD73, CD90 and CD105 and lack expression of CD34, CD45 and HLA-DR.

[0023] Fig. 8 shows the experimental setup for comparison of different carriers. First mesenchymal stem cell population as described herein were outgrown in cell culture flasks. The amount of living mesenchymal stem cells was counted and then 2 million cells/vial were stored for different periods of time in either PlasmaLyte-A or HypoThermosol™-FRS. After storage cells have been counted in sample of $\leq 50\mu\text{l}$ daily for days 1-5 (Total liquid withdrawal $250\mu\text{l}$) and checked for viability by staining the cells with Trypan blue. Further, on days 1, 3 and 5 sample $\leq 80\mu\text{l}$ were taken and analyzed. In addition, the supernatant was obtained and frozen. Then PDGF-AA, PDGF-BB, VEGF, IL-10, Ang-1, HGF and TGF β 1 were measured by FLEXMAP 3D system.

[0024] Fig. 9 summarizes viability data. As can be seen from the left-hand graph, 73 % of the total number of cells (about 95 %) when the storing started were still viable 7 days after storage in HypoThermosol™. On the contrary after 7 days of storage in PlasmaLyte-A only 42 % of the total number of cells (about 94 %) when the storage started were still viable. All counts were based on duplicate readings that are within 10% of one another (following SOP CR D2.600.1). During counting, cells stored in HypoThermosol™ were noticeably smaller with smooth and defined edges. By contrast, cells in Plasmalyte-A appeared in a range of sizes. HypoThermosol™ noticeably supports membrane integrity and presumably survival over a 6 day timespan. Similar results are also shown in the graph of the right-hand side.

[0025] **Fig. 10** shows the results obtained when measuring the cell diameter of cells. The mesenchymal stem cell population as described herein when kept in HypoThermosol™ are narrower in diameter range when compared to cells kept in PlasmaLyte-A. Comparison took place after 3 days of storage.

[0026] **Fig. 11** shows the TGFβ1 concentration in supernatant from the mesenchymal stem cell population as described herein stored in HypoThermosol™ or PlasmaLyte-A after 48 hrs of storage. As can be seen from the graph on the right-hand side, cells secrete about as much TGFβ1 when stored in HypoThermosol™ as when stored in PlasmaLyte-A. In general, over time, the amount of secreted TGFβ1 decreased (graph on the right hand side).

[0027] **Figs. 12 and 13** show control experiments. Here, the PDGF-BB and IL-10 concentrations were measured in supernatant from mesenchymal stem cell populations as described herein stored in HypoThermosol™ or PlasmaLyte-A for 48hrs. Since PDGF-BB or IL-10 are not normally secreted by the mesenchymal stem cell population as described herein, no PDGF-BB or IL-10 were detectable in any sample.

[0028] **Fig. 14** shows the VEGF concentration in supernatant from mesenchymal stem cell populations as described herein stored in HypoThermosol™ or PlasmaLyte-A for 48 hrs. As can be seen from the graph on the right-hand side, cells secrete about as much VEGF when stored in HypoThermosol™ or PlasmaLyte-A on day 0. On day 1 and 5 cells secreted more VEGF when stored in PlasmaLyte-A. Notably, when stored for 3 days cells secreted more VEGF when stored in HypoThermosol™ than when stored in PlasmaLyte-A. Thus, HypoThermosol™ outperforms PlasmaLyte-A by day 3 of storage. The more VEGF detected, the healthier is the culture. Thus, by secreting more VEGF after 3 days storage in HypoThermosol™ than when stored in PlasmaLyte-A, cells were healthier in HypoThermosol™ than in PlasmaLyte-A. From 5 days, storage in PlasmaLyte seems to become more favourable, because at the time point cells stored in PlasmaLyte-A secreted more VEGF. In general, over time, the amount of secreted VEGF decreased (graph on the right hand side).

[0029] **Fig. 15** shows the PDGF-AA concentration in supernatant from mesenchymal stem cell population as described herein stored in HypoThermosol™ or PlasmaLyte-A for 48 hrs. As can be seen from the graph on the right-hand side, cells secrete about as much PDGF-AA when stored in HypoThermosol™ as when stored in PlasmaLyte-A on day 0. On day 1 and 5 cells secreted more PDGF-AA when stored in PlasmaLyte-A. Notably, when stored for 3 days, cells secreted more PDGF-AA when stored in HypoThermosol™ than when stored in

PlasmaLyte-A. Thus, cells stored in HypoThermosol™ are healthier than cells stored in PlasmaLyte-A after 3 days of storage. From 5 days of storage onwards, PlasmaLyte seems to become a more favourable carrier, because at the time point cells stored in PlasmaLyte-A secreted more PDGF-AA. In general, over time, the amount of secreted PDGF-AA decreased (graph on the right hand side).

[0030] **Fig. 16** shows the Ang-1 concentration in supernatant from mesenchymal stem cell populations as described herein stored in HypoThermosol™ or PlasmaLyte-A for 48 hrs. As can be seen from the graph on the right-hand side, cells secrete about as much Ang-1 when stored in HypoThermosol™ or PlasmaLyte-A on day 0 and 3. On day 5 cells secreted more Ang-1 when stored in PlasmaLyte-A. Noticably, when stored for 1 day, cells secreted much more Ang-1 when stored in HypoThermosol™ than when stored in PlasmaLyte-A. Thus, cells stored in HypoThermosol™ seem to be healthier than when stored in PlasmaLyte-A for at least 48 hrs until day 3 of storage. From day 5, PlasmaLyte seems to become a more favourable carrier, because at this time point cells stored in PlasmaLyte-A secreted more Ang-1. In general, over time, the amount of secreted Ang-1 decreased (graph on the right hand side).

[0031] **Fig. 17** shows the HGF concentration in supernatant from mesenchymal stem cell populations as described herein stored in HypoThermosol™ or PlasmaLyte-A after 48 hrs of storage. As can be seen from the graph on the right-hand side, cells secrete about as much HGF when stored in HypoThermosol™ than when stored in PlasmaLyte-A on day 0. On day 3 and 5 cells secreted more HGF when stored in PlasmaLyte-A. Notably when stored for 1 day, cells secreted much more HGF when stored in HypoThermosol™ than when stored in PlasmaLyte-A. Thus, cells stored in HypoThermosol™ seem to be healthier than cells stored in PlasmaLyte-A between at least 1 day (48 hrs) until 3 days of storage. From 3 days onwards PlasmaLyte-A seems to become a more favourable carrier, because at the time points 3 and 5 days, cells stored in PlasmaLyte-A secreted more HGF. In general, over time, the amount of secreted HGF decreased (graph on the right hand side).

[0032] **Fig. 18** are photographs obtained from a preclinical study with the mesenchymal stem cell population of the present invention in pigs. The pigs were rendered diabetic with 120 mg/kg streptozotocin and allowed to recover for 45 days prior to creating six 5 cm x 5 cm full thickness wounds on their backs. Pigs ($n = 2$) were treated twice weekly with 10^5 human mesenchymal stem cell population as described herein per cm^2 for 4 weeks. The two control pigs were treated with PBS. Wounds were photographed on postoperative day 0

(PODay 0) and every seven days until postoperative Day 35. The wounds were analyzed for surface area size by ImageJ. By Day 35, the addition of mesenchymal stem cell population as described herein had resulted in closure of 10 of 12 diabetic wounds (83%), compared to only 3 of 12 (25%) of the PBS-treated control wounds. The *rate* of wound healing was 0.8 cm²/day with the mesenchymal stem cell population as described herein compared to 0.6 cm²/day in the control animals, an improvement of 33%.

[0033] Fig. 19 datasheet of Trolox available from Tocris.

[0034] Fig. 20 shows the datasheet of NaCl available from Sigma Aldrich.

[0035] Fig. 21 shows the datasheet of KH₂PO₄ available from Sigma Aldrich.

[0036] Fig. 22 shows the datasheet for HEPES from Sigma Aldrich.

[0037] Fig. 23 shows the product sheet for sodium lactobionate from COMBI-BLOCKS.

[0038] Fig. 24 shows the product sheet for sucrose from Sigma Aldrich.

[0039] Fig. 25 shows the product sheet for mannitol from avantor.

[0040] Fig. 26 shows the product sheet for glucose from Sigma Aldrich.

[0041] Fig. 27 shows the product sheet for Dextran-40 from Sigma Aldrich.

[0042] Fig. 28 shows the product sheet for adenosine from Sigma Aldrich.

[0043] Fig. 29 shows the product sheet for glutathione from Sigma Aldrich.

[0044] Fig. 30 shows the product sheet for HypoThermosolTM-FRS (HTS-FRS) from STEMCELL Technologies.

DETAILED DESCRIPTION OF THE INVENTION

[0045] As explained above, in a first aspect the invention is directed to a method of transporting/storing a stem cell population, the method comprising transporting/storing said stem cell population contacted with a liquid carrier, said liquid carrier comprising

- i) Trolox;
- ii) Na⁺;
- iii) K⁺;
- iv) Cl⁻;
- v) H₂PO₄⁻;
- vi) HEPES;
- vii) Lactobionate;
- viii) Sucrose;
- ix) Mannitol;

- x) Glucose;
- xi) Dextran-40;
- xii) Adenosine, and
- xiii) Glutathione.

[0046] It has been surprisingly found in the present application that using a liquid carrier as described herein and in particular a liquid carrier such as HypoThermosol™ leads to a superior survival of stem cells compared to other pharmaceutically approved carriers such as e.g. PlasmaLyte®. For example, after 7 days of storage of a mesenchymal stem cell population as described herein in HypoThermosol™ about 70 % of the cells were still viable. On the contrary, after 7 days of storage in PlasmaLyte® only about 40 % of the cells were still viable (see Examples, when measured with a hemocytometer). Thus, using a liquid carrier as described herein allows the transport/storage of stem cells over a period of time without substantial loss of the viability of cells. In particular, storage in HypoThermosol™ for shorter time period of 3 days or less seems to be especially beneficial, since the stem cells in general secreted more factors than after storage in PlasmaLyte-A as described in the Experimental Section in detail.

[0047] When used herein the term ‘transport’ or ‘transporting’ any transport is meant. Such transport may be performed with any vehicle, such as car, train, and airplane or by a person carrying/transporting a container comprising the stem cells contacted with the liquid carrier from one place to another place. In one embodiment, transporting is carried out from the place of production of the stem cell population of interest to the place of stem cell administration (for example, the GMP facility in which a stem cell population of interest is produced to the site of administration of the stem cell population, for example, a clinic or a doctor’s office). It is however also envisioned that the term ‘transporting relates to a storage of cells at the same place for a period of time. For example, stem cells may be stored after harvest until their application to a subject at one place. The container in which the stem cells can be stored or transported can be any container suitable for the method of the present invention.

[0048] The transporting/storing can be performed for any period of time. For example, the transporting/storing can be performed for about 7 days or less. It is also envisioned that the transporting/storing can be performed for about 6, 5, 4, 3, 2, 1, day(s) or less. It can thus be that the transporting/storing is performed for about 48 hours or about 24 hours or less.

[0049] It is also contemplated that the transporting/storing is performed at any temperature suitable for the method of the present invention. For example, the transporting/storing can be performed at a temperature of about -5°C to about 15°C. It is therefore also envisioned that the transporting/storing can be performed at a temperature of about 2°C to about 8°C. The transporting can also be carried out at a temperature of more than about -5°C, more than about -10°C, more than about -15°C, or more than about -20°C. Further it is envisioned that transporting/storing can be performed at a temperature of below 20°C, below 18 °C, below 15 °C, below 12 °C or below 10 °C.

[0050] The method of the present invention also envisions that the stem cell population is stored or transported in any suitable concentration. The stem cell population may, for example, be transported/stored in a concentration of about 70 million cells per 1 ml carrier, of about 60 million cells million cells per 1 ml carrier, of about 50 million cells per 1 ml carrier, of about 40 million cells per 1 ml carrier, of about 30 million cells per 1 ml carrier, of about 20 million cells per 1 ml carrier, of about 10 million cells per 1 ml carrier, of about 5 million cells per 1 ml carrier, of about 4 million cells per 1 ml carrier, of about 3 million cells per 1 ml carrier, of about 2 million cells per 1 ml carrier, of about 1 million cells per 1 ml carrier, of about 0.5 million cells per 1 ml carrier, of about 0.1 million cells per 1 ml carrier or of less than 0.1 million cells per 1 ml carrier. Therefore, the stem cell population can be transported/stored in a concentration of about 10 million cells per ml carrier to about 1 million cells per 1 ml carrier.

[0051] The method of the present invention concerns the transporting/storing of stem cells. In principle, any stem cell can be used in the method of the present invention. One characterizing feature of stem cells is their ability to self-renew. 'Self-renewal' is the ability to go through numerous cell cycles of cell division while maintaining the undifferentiated state. Methods for testing if a cell has the capacity to self-renew are known to the skilled artisan. For example, self-renewal may be tested by passaging the cells over more than 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more passages. Passaging includes splitting of the cells before re-plating them as a single cell suspension. A further characteristic of stem cells is their multipotency or pluripotency as will also be described elsewhere herein. In principle, multipotency or pluripotency can be tested by differentiating said stem cells into different lineages.

[0052] In particular, the stem cell population used in the method of the present invention can be an embryonic stem cell population, an adult stem cell population, a mesenchymal stem cell population or an induced pluripotent stem cell population.

[0053] As used herein an "embryonic stem cell population" is a "pluripotent stem cell population". A pluripotent cell when referred to herein relates to a cell type having the capacity for self-renewal, and the potential of differentiation into different cell types. Pluripotent stem cells can differentiate into nearly all cells, i.e. cells derived from any of the three primary germ layers: ectoderm, endoderm, and mesoderm. The term pluripotent stem cell also encompasses stem cells derived from the inner cell mass of an early stage embryo known as a blastocyst. Notably, recent advances in embryonic stem cell research have led to the possibility of creating new embryonic stem cell lines without destroying embryos, for example by using a blastomere biopsy-based technique, which does not interfere with the embryo's developmental potential (Klimanskaya (2006) "Embryonic stem cells from blastomeres maintaining embryo viability." *Semin Reprod Med.* 2013 Jan;31(1):49-55). Furthermore, a large number of established embryonic stem cell lines are available in the art. Thus, it is possible to work with embryonic stem cells without the necessity to destroy an embryo. The pluripotent stem cells can be embryonic stem cells, which have not been obtained via the destruction of a human embryo. Thus, the pluripotent stem cells are embryonic stem cells obtained from an embryo, without the destruction of the embryo.

[0054] As used herein an "adult stem cell population" is a multipotent stem cell population. A multipotent stem cell population can give rise a restricted number of cell types, therefore they are somatic fate restricted. For example, a neural stem cell can give rise to both neuronal and glial cells. Adult stem cells have the capability to self-renew and may be obtained from any suitable source. For example, adult stem cells may be obtained from bone marrow, peripheral blood, brain, spinal cord, dental pulp, blood vessels, skeletal muscle, epithelia of the skin and digestive system, cornea, retina, liver, or pancreas.

[0055] The stem cell population used in the method of the present invention may also be a mesenchymal stem cell population. In this context, it is noted that the culture medium described herein (e.g. PTT-6) allows the isolation of a mesenchymal stem cell population (also referred herein as "mesenchymal stem cells") from the amniotic membrane under conditions that allow cell proliferation of the mesenchymal stem/progenitor cells without differentiation of the mesenchymal stem/progenitor cells. Thus, after isolation of the mesenchymal stem cells from the amniotic membrane as described herein the isolated

mesenchymal stem/progenitor cell population has the capacity to differentiate into multiple cell types as described in US patent application 2006/0078993, US patent 9,085,755, International patent application WO2006/019357, US patent 8,287,854 or WO2007/046775, for instance. As described in US patent application 2006/0078993, for example, the mesenchymal stem cells of the amniotic membrane of the umbilical cord have a spindle shape, express the following genes: POU5f1, Bmi-1, leukemia inhibitory factor (LIF), and secrete Activin A and Follistatin. The mesenchymal stem cells isolated in the present invention can, for example, be differentiated into any type of mesenchymal cell such as, but not limited to, adipocytes, skin fibroblasts, chondrocytes, osteoblasts, tenocytes, ligament fibroblasts, cardiomyocytes, smooth muscle cells, skeletal muscle cells, mucin producing cells, cells derived from endocrine glands such as insulin producing cells (for example, β -islet cells) or neurectodermal cells. The stem cells isolated in accordance with the method described herein can be differentiated *in vitro* in order to subsequently use the differentiated cell for medical purposes. An illustrative example of such an approach is the differentiation of the mesenchymal stem cells into insulin producing β -islet cells which can then be administered, for example by implantation, to a patient that suffers from an insulin deficiency such as diabetes mellitus (cf. also WO2007/046775 in this respect). Alternatively, the mesenchymal stem cells described herein can be used in their undifferentiated state for cell-based therapy, for example, for wound healing purposes such as treatment of burns or chronic diabetic wounds. In these therapeutic applications the mesenchymal stem cells of the invention can either serve to promote wound healing by interacting with the surrounding diseased tissue or can also differentiate into a respective skin cell (cf., again WO2007/046775, for example).

[0056] In this context, it is noted that the mesenchymal stem cell population described herein can be isolated and cultivated (i.e. are derived) from any umbilical cord tissue as long as the umbilical cord tissue contains the amniotic membrane (which is also referred to as “cord lining”). Accordingly, the mesenchymal stem cell population can be isolated from (pieces of) the entire umbilical cord as described in the Experimental section of the present application. This umbilical cord tissue may thus contain, in addition to the amniotic membrane, any other tissue/component of the umbilical cord. As shown, for example, in Figure 16 of US patent application 2006/0078993 or International patent application WO2006/019357, the amniotic membrane of the umbilical cord is the outermost part of the umbilical cord, covering the cord. In addition, the umbilical cord contains one vein (which

carries oxygenated, nutrient-rich blood to the fetus) and two arteries (which carry deoxygenated, nutrient-depleted blood away from the fetus). For protection and mechanical support these three blood vessels are embedded in Wharton's jelly, a gelatinous substance largely of mucopolysaccharides. Accordingly, the umbilical cord tissue used herein can also comprise this one vein, the two arteries and the Wharton's jelly. The use of such an entire (intact) section of the umbilical cord has the advantage that the amniotic membrane does not need to be separated from the other components of the umbilical cord. This reduces the isolation steps and thus makes the method described herein, simpler, faster, less error prone and more economical – which are all important aspects for the GMP production that is necessary for therapeutic application of the mesenchymal stem cells. The isolation of the mesenchymal stem cells can thus start by tissue explant, which may be followed by subsequent subculturing (cultivation) of the isolated mesenchymal stem cells if greater amounts of the mesenchymal stem cells are desired, for example, for use in clinical trials. Alternatively, it is also possible to first separate the amniotic membrane from the other components of the umbilical cord and isolate the mesenchymal cord lining stem cells from the amniotic membrane by cultivation of the amniotic membrane in a culture medium e.g. PTT-6. This cultivation can also be carried out by tissue explant, optionally followed by subculturing of the isolated mesenchymal stem cells. In this context, the term “tissue explant” or “tissue explant method” is used in its regular meaning in the art to refer a method in which a tissue, once being harvested, or a piece of the tissue is being placed in a cell culture dish containing culture (growth) medium and by which over time, the stem cells migrate out of the tissue onto the surface of the dish. These primary stem cells can then be further expanded and transferred into fresh dishes through micropropagation (subculturing) as also described here. In this context, it is noted that in terms of production of the cells for therapeutic purposes, in the first step of isolating the amniotic membrane mesenchymal stem cells from the umbilical cord, a master cell bank of the isolated mesenchymal stem cells is obtained, while with the subsequent subculturing, a working cell bank can be obtained. In particular embodiments, the stem cell population thus is a mesenchymal stem cell population.

[0057] The mesenchymal stem cell population may be isolated from the amniotic membrane of the umbilical cord by a method comprising cultivating umbilical cord tissue in a culture medium comprising DMEM (Dulbecco's modified eagle medium), F12 (Ham's F12 Medium), M171 (Medium 171) and FBS (Fetal Bovine Serum). Using such a medium provides for the isolation of a mesenchymal stem cell population from the amniotic

membrane of the umbilical cord of which more than 90 %, or even 99 % or more of the cells are positive for the three mesenchymal stem cell markers CD73, CD90 and CD105 while at the same these stem cells lack expression of CD34, CD45 and HLA-DR (see the Experimental Section), meaning 99 % or even more cells of this population express the stem cell markers CD73, CD90 and CD105 while not expressing the markers CD34, CD45 and HLA-DR . Such an extremely homogenous and well defined cell population has been reported for the first time in co-pending US application Serial No. 15/725,913, filed 5 October 2018 claiming priority to US provisional application Serial No. 62/404,582 filed 5 October 2017, the content of both of which is incorporated by reference herein in its entirety) and as well as in co-pending PCT application PCT/SG2017/050500 also filed 5 October 2018 claiming priority to US provisional application No. 62/404,582 filed 5 October 2017 and is the ideal candidate for clinical trials and cell based therapies since, this stem cell population for example, fully meets the criteria generally accepted for human mesenchymal stem cells to be used for cellular therapy as defined, for example, by Dominici et al, “Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement”, *Cytotherapy* (2006) Vol. 8, No. 4, 315-317, Sensebe et al., “Production of mesenchymal stromal/stem cells according to good manufacturing practices: a review”, *Stem Cell Research & Therapy* 2013, 4:66), Vonk et al., *Stem Cell Research & Therapy* (2015) 6:94, or Kundrotas *Acta Medica Lituanica*. 2012. Vol. 19. No. 2. P. 75–79. Also, using a bioreactor such as a Quantum Cell Expansion System, it is possible to obtain high numbers of mesenchymal stem cells such as 300 to 700 million mesenchymal stem cells per run (see also the Experimental Section). Thus, the present invention allows transporting/storing amounts of stem cells that are needed for therapeutic applications, such as their use in wound healing, in a cost efficient manner. In addition, all components used for making the culture medium of the present invention are commercially available in GMP quality. Accordingly, the present invention opens the route to transport/store a GMP produced and highly homogenous mesenchymal stem cell population from the amniotic membrane of the umbilical cord.

[0058] Thus, in some embodiments the mesenchymal stem cell population is an isolated mesenchymal stem population of the amniotic membrane of the umbilical cord. It is further envisioned that at least about 90 % or more cells of the isolated mesenchymal stem cell population express each of the following markers: CD73, CD90 and CD105. For example, at least about 91 % or more, about 92 % or more, about 93 % or more, about 94 % or more,

about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more cells of the isolated mesenchymal stem cell population express each of CD73, CD90 and CD105. Additionally or alternatively, at least about 90 % or more, about 91 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more of the isolated mesenchymal stem cells lack expression of the following markers: CD34, CD45 and HLA-DR (Human Leukocyte Antigen – antigen D Related).

[0059] The marker CD73 is known to the skilled person. In this regard CD73 refers to cluster of differentiation 73 also known as 5'-nucleotidase (5'-NT) or ecto-5'-nucleotidase. The sequence of the human CD73 protein may have the sequence of SEQ ID NO. 1. The marker CD90 is known to the skilled person. In this regard CD90 refers to Cluster of Differentiation 90 also known as Thymocyte differentiation antigen 1 (Thy-1). The sequence of the human CD90 protein may have the sequence of SEQ ID NO: 2. The marker CD105 is known to the skilled person. CD105 is also known as Endoglin (ENG). The sequence of the human CD105 protein may have the sequence of SEQ ID NO: 3.

[0060] If a mesenchymal stem cell population of the invention (in particular a population of the mesenchymal stem cells of which at least about 98% or 99 % or express each of the markers CD73, CD90 and CD105 and lack expression of each of the markers: CD34, CD45 and HLA-DR) is used for clinical trials or as an approved therapeutic, a cell population of the working cell bank will typically be used for this purpose. As explained, the mesenchymal stem cell population may lack expression of the following markers: CD34, CD45 and HLA-DR. In this context it is noted that the marker CD34, CD45 and HLA-DR are known to the skilled person. The human CD34 protein may have the sequence of SEQ ID NO. 4. The human CD45 protein may have the sequence of SEQ ID NO: 5. The human HLA-DR protein may have the sequence of SEQ ID NO: 6.

[0061] Both the stem cell population of the isolation step (which may make up the master cell bank) and the stem cell population of the subculturing step (which may make up the working cell bank) can, for example, be stored in cryo-preserved form.

[0062] As mentioned above, the present method of isolating mesenchymal stem cells from the amniotic membrane of umbilical cord has the advantage that all components used in the culture medium of the invention are available in GMP quality and thus provide the possibility to isolate the mesenchymal stem cells under GMP conditions for subsequent therapeutic administration.

[0063] Thus, the stem cell population can also be an induced pluripotent stem cell population. "Induced pluripotent stem cells", as used herein, refer to adult somatic cells that have been genetically reprogrammed to an embryonic stem cell–like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells. Thus, induced pluripotent stem cells can be derived/generated from a non-pluripotent cell.

[0064] Induced pluripotent stem cells are an important advancement in stem cell research, as they allow obtaining pluripotent stem cells without the use of embryos. Mouse iPSCs were first reported in 2006 (Takahashi, K; Yamanaka, S (2006). "Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors". *Cell* 126 (4): 663–76), and human iPSCs (hiPSCs) were first reported in 2007 (Takahashi et al. (2007) "Induction of pluripotent stem cells from adult human fibroblasts by defined factors." *Cell*; 131(5):861-72). Mouse iPSCs demonstrate important characteristics of pluripotent stem cells, including expression of stem cell markers, forming tumors containing cells from all three germ layers, and being able to contribute to many different tissues when injected into mouse embryos at a very early stage in development. Human iPSCs also express stem cell markers and are capable of generating cells characteristic of all three germ layers. Such stem cell markers can include Oct3/4, Sox2, Nanog, alkaline phosphatase (ALP) as well as stem cell-specific antigen 3 and 4 (SSEA3/4). Also, the chromatin methylation patterns of iPSC are similar to that of embryonic stem cells (Tanabe, Takahashi, Yamanaka (2014) "Induction of pluripotency by defined factors." *Proc. Jpn. Acad.*, 2014, Ser. B 90).

[0065] In addition, iPSCs are able to self-renew *in vitro* and differentiate into all three germ layers. The pluripotency or the potential to differentiate into different cell types of iPSC can be tested, e.g., by *in vitro* differentiation into neural or glia cells or the production of germline chimeric animals through blastocyst injection.

[0066] Methods for the generation of human induced pluripotent stem cells are well known to the skilled person and for example described in WO2009115295, WO2009144008 or EP2218778. Thus, the skilled artisan can obtain an iPSC by any method. In principle, induced pluripotent stem cells may be obtained from any adult somatic cell (of a subject). Exemplary somatic cells include peripheral blood Mononuclear Cells (PBMCs) from blood or fibroblasts obtained from skin tissue biopsies.

[0067] The method of the present invention includes that the stem cell population as described herein is contacted with a liquid carrier. It is envisioned that in the method of the

present invention the stem cell population as described herein is contacted with the carrier before transporting/storing. Additionally or alternatively, the stem cell population is contacted with the carrier after its harvest. How harvesting can be performed is described in detail elsewhere herein as well as in the Experimental Section. For example, the stem cell population can be contacted with the carrier about 0 minutes, about 1 minute, about 5 minutes, about 10 minutes, about 30 minutes, about 45 minutes, about 60 minutes or a longer time after its harvest.

[0068] Harvesting can comprise separating the stem cell population from culture medium e.g. from PTT-6. Suitable techniques for such separation are known to the skilled person. For example, separating can be performed by centrifuging the stem cells within a culture medium and decanting the culture medium.

[0069] The stem cell population is contacted with a liquid carrier, wherein the liquid carrier comprises

- i) Trolox;
- ii) Na^+ ;
- iii) K^+ ;
- iv) Cl^- ;
- v) H_2PO_4^- ;
- vi) HEPES;
- vii) Lactobionate;
- viii) Sucrose;
- ix) Mannitol;
- x) Glucose;
- xi) Dextran-40;
- xii) Adenosine, and
- xiii) Glutathione.

[0070] By “Trolox” is meant 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid of CAS Number 53188-07-1. It is a water-soluble analog of vitamin E and is suggested to reduce oxidative stress or damage. Fig. 19 shows the datasheet of Trolox available from Tocris. It also commercially available from Sigma Aldrich (product number: 238813).

[0071] Both of Na^+ and Cl^- are well known ions. The skilled person knows how to obtain these. For example, these ions may be added to the carrier as a NaCl salt. NaCl in GMP

quality can be obtained from Sigma Aldrich. Fig. 20 shows the datasheet of NaCl available from Sigma Aldrich.

[0072] K^+ and $H_2PO_4^-$ (dihydrogen phosphate) are also well known to the skilled person. It may be used e.g. as a KH_2PO_4 obtainable from SigmaAldrich. Fig. 21 shows the datasheet of KH_2PO_4 available from Sigma Aldrich.

[0073] HEPES also named 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (CAS Number 7365-45-9) is commonly used as a zwitterionic organic chemical buffering agent. The person skilled in the art also knows where to obtain HEPES, which is commercially available. For example, she/he may obtain it from Sigma Aldrich; the corresponding data sheet shown in Fig. 22.

[0074] Lactobionate is the carboxylate anion of lactobionic acid. Lactobionic acid (4-*O*- β -galactopyranosyl-D-gluconic acid) is a sugar acid. Lactobionate can be used in different ways. When used as potassium lactobionate it can e.g. provide osmotic support and prevent cell swelling and when combined with sodium it may have a preservative function. Alternatively, mineral salts of lactobionic acid can be used for mineral supplementation. For pharmaceutical applications, often the antibiotic erythromycin can inter alia be used as the salt erythromycin lactobionate. The skilled person also knows where to obtain lactobionate e.g. sodium lactobionate (Cas Number: 27297-39-8), namely from e.g. COMBI-BLOCKS, see product sheet in Fig. 23.

[0075] Sucrose , also known as D-Glc-(1 \rightarrow 2)- β -D-Fru, α -D-glucopyranosyl β -D-fructofuranoside, β -D-fructofuranosyl- α -D-glucopyranoside, D(+)-saccharose or sugar (CAS Number 57-50-1) can as the other substances be commercially obtained and the skilled person knows where to buy it as well. The corresponding product sheet for sucrose from Sigma Aldrich is shown in Fig. 24.

[0076] Mannitol is a type of sugar alcohol (CAS Registry Number: 69-65-8). The person skilled in the art knows how to obtain mannitol. For example, it may be obtained from Avantor. The respective product sheet is shown in Fig. 25.

[0077] Glucose (CAS Number: 50-99-7) is also well known to the skilled person and commercially available. A respective product sheet from Sigma Aldrich is shown in Fig. 26.

[0078] Dextran is a branched glucan composed of linear α (1 \rightarrow 6) linked glucose units and α (1 \rightarrow 3) link initiated branches. Dextran ranges in size from 10,000 to 150,000 Kd. Dextran are used in many applications as volume extenders, stabilizers, matrix components, binding platforms, lubricants and physical structure components. Dextran 40 (CAS Number: 9004-

54-0) as used in the carrier described herein is typically used in the development of new improved preservation solutions for organ transplantation. Dextran 40 may be used to determine cell tightness and flux parameters across cell layers. Dextran 40 can also be used as a colloidal plasma volume extender. Dextran-40 is commercially available and can inter alia be obtained from Sigma Aldrich (product sheet shown in Fig. 27).

[0079] Adenosine (CAS Number 58-61-7) is a purine nucleoside composed of a molecule of adenine attached to a ribose sugar molecule (ribofuranose) moiety via a β -N₉-glycosidic bond. Adenosine is commercially available inter alia from Sigma-Aldrich (the corresponding product sheet is shown in Fig. 28).

[0080] Glutathione is also known as (2S)-2-Amino-4-[[[(1R)-1-[(carboxymethyl)carbamoyl]-2-sulfanylethyl]carbamoyl]butanoic acid. This component is commercially available and can inter alia be obtained from Sigma Aldrich (corresponding product sheet shown in Fig. 29).

[0081] In principle any liquid carrier comprising the substances as listed in i)-xiii) above can be used in the method of the present invention. The carrier is a liquid carrier. Thus, it is possible that the substances as listed in i)-xiii) are dissolved in a liquid to form a solution/suspension. The liquid may be any suitable liquid. For example, the liquid can be a culture medium, water, buffer, or the like.

[0082] The carrier may additionally comprise further pH buffers, energy substrates, free radical scavengers, and osmotic/oncotic stabilizers – all known to the skilled person. Furthermore, the liquid carrier may be serum-free and/or protein-free. The liquid carrier may not comprise a dipolar aprotic solvent such as for example DMSO. In particular, the liquid carrier may be a carrier as described in WO 2010/064054. The carrier may be HypoThermosol™ or HypoThermosol™-FRS (HTS-FRS). HypoThermosol™-FRS (HTS-FRS) can be purchased from STEMCELL Technologies (according to the respective product sheet shown in Fig. 30).

[0083] It is further envisioned that the carrier is a transport/storage medium or an excipient. A transport/storage medium, may be a natural medium, which consists solely of naturally occurring biological fluids, which additionally comprise substances as listed in i)-xiii) as described herein. The medium can also be one comprising substances as listed in i)-xiii) as described herein and addition of (further) nutrients (both organic and inorganic), vitamins, salts, O₂ and CO₂ gas phases, serum proteins, carbohydrates, and/or cofactors. In particular embodiments the medium is serum and/or protein free.

[0084] The carrier may also be an excipient. An “excipient” is a substance formulated alongside the active ingredient of a medication. In the present method the active ingredient is the stem cell population.

[0085] The carrier may further comprise biocompatible scaffolds or microcarriers. The scaffolds or microcarriers can, for example, be biodegradable polymeric substances, most preferably poly(D,L lactic-co-glycolic acid) (PLGA)). Alternatively, the scaffolds or microcarriers may be smooth, macroporous or microporous structures comprising substances including poly-L-lactide (PLLA), collagen, fibronectin, glycosaminoglycans (GAGs), fibrin, starch, cellulose arabinogalactan (larch gum), alginic acid, agar, carrageenan, chitin, hyaluronic acid, dextran, gellan gum, pullulan, hydroxyapatite, polyhydroxyalkanoates (PHAs), hydrogels or other self-assembling materials such as peptide based nanostructured fibrous scaffolds.

[0086] In principle any amount of stem cells can be contacted with any amount of liquid carrier. In this regard the contacting can be performed by suspending the stem cell population in a density of about 70 million/ml, of about 60 million/ml, of about 50 million/ml, of about 40 million/ml, of about 30 million/ml, of about 20 million/ml, of about 10 million/ml, of about 5 million/ml, of about 4 million/ml, of about 3 million/ml, of about 2 million/ml, of about 1 million/ml, of about 0.5 million/ml, of about 0.1 million/ml or of less than 0.1 million cells in 1 ml of the carrier. In some embodiments, the contacting is performed by suspending the stem cell population in a density of about 10 million/1 ml carrier.

[0087] After contacting the stem cell population with the carrier, the stem cells contacted with the carrier can be aliquoted into vials in a volume of about 50 ml, of about 20 ml, of about 10 ml, of about 5 ml, of about 4 ml, of about 3 ml, of about 2 ml, of about 1 ml, of about 0.5 ml, of about 0.25 ml or of less than 0.25 ml carrier. For example, the stem cells that have been contacted with the carrier can be aliquoted into vials in a volume of about 1 ml.

[0088] It is further envisioned that the method of the present invention does not comprise a thawing or freezing step. This may include that after their harvest the stem cell population is transported/stored without the need to freeze and thaw the stem cell population.

[0089] The carrier used in the method of transporting/storing the stem cell population as described herein is particularly suited for this purpose. One advantage of this carrier is that substantially all stem cells transported/stored therein remain viable. A “viable cell” is a cell

able to live. The person skilled in the art knows how to detect viable cells. One such method is staining cells with the dye Trypan blue. Viable cells do not stain positive with Trypan blue.

[0090] In this regard, in the method of the present invention at most about 50 %, about 40 %, about 30 %, about 20 %, about 10 % or less than about 10 % of the stem cells of the population may die during transporting/storing compared to the number/amount of viable stem cells before transporting/ storing.

[0091] The method of the present invention also contemplates that the stem cell population has any cell diameter after transporting/storage. The person skilled in the art knows how to measure the diameter of a cell. For example, cell size/diameter may be determined by capturing a microscope image and using secondary software to measure the diameter of the cell. Most of the stem cells in the stem cell population can therefore have a cell diameter between about 9 μm and about 20 μm after transporting/storage. It is also envisioned that most of the stem cells in the stem cell population have a cell diameter between about 12 μm and about 16 μm after transporting.

[0092] The stem cells transported/stored in the carrier as described herein secrete the same proteins/ factors as viable stem cells. For example, the method of the present invention contemplates that after transport/storage the (mesenchymal) stem cell population may secrete about as much TGFbeta 1 as before transporting/storage. TGFbeta 1 (Transforming growth factor beta, TGF- β 1) is known to the skilled person and may comprise the sequence as shown in SEQ ID NO. 7. Additionally or alternatively, after transporting/storing the (mesenchymal) stem cell population may secrete about as much VEGF (Vascular endothelial growth factor), PDGF-AA (Platelet-derived growth factor subunit AA), Ang-1 (Angiogenin-1), and/or HGF (Hepatocyte growth factor) as before transporting/storing. All of VEGF, PDGF-AA, Ang-1, and/or HGF are known to the skilled person for their involent in wound healing. In particular, VEGF may comprise a sequence as shown in SEQ ID NO. 8, PDGF-AA may have a sequence as shown in SEQ ID NO. 9, Ang-1 may have a sequence as shown in SEQ ID NO. 10 while HGF may have a sequence as shown in SEQ ID NO. 11. Additionally or alternatively, essentially no PDGF-BB and/or IL-10 is detected before and/or after transporting. Both of PDGF-BB (Platelet-derived growth factor subunit BB) and/or IL-10 (interleukin-10) are also known to the skilled person. PDGF-BB may comprise a sequence as shown in SEQ ID NO. 12 while IL-10 may comprise a sequence as shown in SEQ ID NO: 13. The secretion of these factors can be determined with any suitable method, for example, by measuring the amount of protein (i.e., for example, PDGF-AA, PDGF-BB, VEGF, IL-10,

Ang-1, HGF or TGF β 1) that the stem cells secrete into the carrier. The amount of protein can be measured by commercially available antibodies/immunoassays in an automated fashion, using, for example a system such as the FLEXMAP 3D system (Luminex Corporation, Austin, Texas, USA). In this context, it is noted that involvement of the proteins Angiopoietin 1 (Ang-1), TGF- β 1, VEGF, and HGF in the wound healing process is known to the person skilled in the art. For the involvement of Angiopoietin 1 in wound healing, see, for example, Li et al. *Stem Cell Research & Therapy* 2013, 4:113 “Mesenchymal stem cells modified with angiopoietin-1 gene promote wound healing”. For the involvement of Hepatocyte Growth Factor (HGF) in wound healing, in particular healing of chronic/non-healing wounds see for example, Yoshida et al., “Neutralization of Hepatocyte Growth Factor Leads to Retarded Cutaneous Wound Healing Associated with Decreased Neovascularization and Granulation Tissue Formation” *J. Invest. Dermatol.* 120:335-343, 2003, Li, Jin-Feng et al. “HGF Accelerates Wound Healing by Promoting the Dedifferentiation of Epidermal Cells through β 1-Integrin/ILK Pathway.” *BioMed Research International* 2013 (2013): 470418 or Conway et al., “Hepatocyte growth factor regulation: An integral part of why wounds become chronic” *Wound Rep Reg* (2007) 15 683–692. For the involvement of Vascular Endothelial Growth Factor (VEGF) in wound healing, in particular healing of chronic/non-healing wounds, see for example Froget et al., *Eur. Cytokine Netw.*, Vol. 14, March 2003, 60–64 or Bao et al., “The Role of Vascular Endothelial Growth Factor in Wound Healing” *J Surg Res.* 2009 May 15; 153(2): 347–358.

[0093] For the involvement of Transforming Growth Factor Beta (including TGF- β 1, TGF- β 2, and TGF- β 3) in wound healing, in particular healing of chronic/non-healing wounds see for example, Ramirez et al. “The Role of TGF β Signaling in Wound Epithelialization” *Advances In Wound Care*, Volume 3, Number 7, 2013, 482-491 or Pakyari et al., *Critical Role of Transforming Growth Factor Beta in Different Phases of Wound Healing*, *Advances In Wound Care*, Volume 2, Number 5, 2012, 215-224.

[0094] Turning now to the culture medium used in the present invention, the culture medium may comprise, for the isolation or cultivation of the mesenchymal cord lining stem cells, DMEM in a final concentration of about 55 to 65 % (v/v), F12 in a final concentration of about 5 to 15 % (v/v), M171 in a final concentration of about 15 to 30 % (v/v) and FBS in a final concentration of about 1 to 8 % (v/v). The value of “% (v/v)” as used herein refers to the volume of the individual component relative to the final volume of the culture medium.

This means, if DMEM is, for example, present in the culture medium at a final concentration of about 55 to 65 % (v/v), 1 liter of culture medium contains about 550 to 650 ml DMEM.

[0095] In other embodiments, the culture medium may comprise DMEM in a final concentration of about 57.5 to 62.5 % (v/v), F12 in a final concentration of about 7.5 to 12.5 % (v/v), M171 in a final concentration of about 17.5 to 25.0 % (v/v) and FBS in a final concentration of about 1.75 to 3.5 % (v/v). In further embodiments, the culture medium may comprise DMEM in a final concentration of about 61.8 % (v/v), F12 in a final concentration of about 11.8 % (v/v), M171 in a final concentration of about 23.6 % (v/v) and FBS in a final concentration of about 2.5 % (v/v).

[0096] In addition to the above-mentioned components, the culture medium may comprise supplements that are advantageous for cultivation of the mesenchymal cord lining stem cells. The culture medium of the present invention may, for example, comprise Epidermal Growth Factor (EGF). If present, EGF may be present in the culture medium in a final concentration of about 1 ng/ml to about 20 ng/ml. In some of these embodiments, the culture medium may comprise EGF in a final concentration of about 10ng/ml.

[0097] The culture medium may also comprise insulin. If present, insulin may be present in a final concentration of about 1 µg/ml to 10 µg/ml. In some of these embodiments, the culture medium may comprise Insulin in a final concentration of about 5µg/ml.

[0098] The culture medium may further comprise at least one of the following supplements: adenine, hydrocortisone, and 3,3',5-Triiodo-L-thyronine sodium salt (T3). In such embodiments, the culture medium may comprise all three of adenine, hydrocortisone, and 3,3',5-Triiodo-L-thyronine sodium salt (T3). In these embodiments, the culture medium may comprise adenine in a final concentration of about 0.05 to about 0.1 µg/ml, hydrocortisone in a final concentration of about 1 to about 10 µg/ml and/or 3,3',5-Triiodo-L-thyronine sodium salt (T3) in a final concentration of about 0.5 to about 5 ng/ml.

[0099] In one embodiment, the mesenchymal stem cells are cultured in PTT6 medium to obtain the highly purified mesenchymal stem cell population described and used herein. In this context it is noted that PTT6 medium as described herein is obtained by mixing to obtain a final volume of 500 ml culture medium:

- i. 250 ml of DMEM
- ii. 118 ml M171
- iii. 118 ml DMEM/F12

- iv. 12.5 ml Fetal Bovine Serum (FBS) to reach a final concentration of 2.5% (v/v)
- v. EGF in a final concentration of 10ng/ml
- vi. Insulin in a final concentration of 5µg/ml.
- vii. Insulin 0.175 ml (final concentration of 5µg/ml)

[00100] By “DMEM” is meant Dulbecco’s modified eagle medium which was developed in 1969 and is a modification of basal medium eagle (BME) (cf. Fig.1 showing the data sheet of DMEM available from Lonza). The original DMEM formula contains 1000 mg/L of glucose and was first reported for culturing embryonic mouse cells. DMEM has since then become a standard medium for cell culture that is commercially available from various sources such as ThermoFisher Scientific (catalogue number 11965-084), Sigma Aldrich (catalogue number D5546) or Lonza, to name only a few suppliers. Thus, any commercially available DMEM can be used in the present invention. In preferred embodiments, the DMEM used herein is the DMEM medium available from Lonza under catalog number 12-604F. This medium is DMEM supplemented with 4.5 g/L glucose and L-glutamine. In another preferred embodiment the DMEM used herein is the DMEM medium of Sigma Aldrich catalogue number D5546 that contains 1000 mg/L glucose, and sodium bicarbonate but is without L-glutamine.

[00101] By “F12” medium is meant Ham’s F12 medium. This medium is also a standard cell culture medium and is a nutrient mixture initially designed to cultivate a wide variety of mammalian and hybridoma cells when used with serum in combination with hormones and transferrin (cf. Fig. 2, showing the data sheet of Ham’s F12 medium from Lonza). Any commercially available Ham’s F12 medium (for example, from ThermoFisher Scientific (catalogue number 11765-054), Sigma Aldrich (catalogue number N4888) or Lonza, to name only a few suppliers) can be used in the present invention. In preferred embodiments, Ham’s F12 medium from Lonza is used.

[00102] By “DMEM/F12” or “DMEM:F12” is meant a 1:1 mixture of DMEM with Ham’s F12 culture medium (cf. Fig. 3 showing the data sheet for DMEM: F12 (1:1) medium from Lonza). DMEM/F12 (1:1) medium is a widely used basal medium for supporting the growth of many different mammalian cells and is commercially available from various suppliers such as ThermoFisher Scientific (catalogue number 11330057), Sigma Aldrich (catalogue number D6421) or Lonza. Any commercially available DMEM:F12 medium can be used in the present invention. In preferred embodiments, the DMEM:F12 medium used

herein is the DMEM/F12 (1:1) medium available from Lonza under catalog number 12-719F (which is DMEM: F12 with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose).

[00103] By “M171” is meant culture medium 171, which has been developed as basal medium for the culture and growth of normal human mammary epithelial cells (cf. Fig. 4 showing the data sheet for M171 medium from Life Technologies Corporation). This basal medium is widely used and is commercially available from suppliers such as ThermoFisher Scientific or Life Technologies Corporation (catalogue number M171500), for example. Any commercially available M171 medium can be used in the present invention. In preferred embodiments, the M171 medium used herein is the M171 medium available from Life Technologies Corporation under catalogue number M171500.

[00104] By “FBS” is meant fetal bovine serum (that is also referred to as “fetal calf serum”), i.e. the blood fraction that remains after the natural coagulation of blood, followed by centrifugation to remove any remaining red blood cells. Fetal bovine serum is the most widely used serum-supplement for *in vitro* cell culture of eukaryotic cells because it has very low level of antibodies and contains more growth factors, allowing for versatility in many different cell culture applications. The FBS is preferably obtained from a member of the International Serum Industry Association (ISIA) whose primary focus is the safety and safe use of serum and animal derived products through proper origin traceability, truth in labeling, and appropriate standardization and oversight. Suppliers of FBS that are ISIA members include Abattoir Basics Company, Animal Technologies Inc., Biomin Biotechnologia LTDA, GE Healthcare, Gibco by Thermo Fisher Scientific and Life Science Production, to mention only a few. In currently preferred embodiments, the FBS is obtained from GE Healthcare under catalogue number A15-151.

[00105] As mentioned above, a method of making a culture medium for isolating the mesenchymal stem cell population used in the invention comprises mixing to obtain a final volume of 500 ml culture medium:

- i. 250 ml of DMEM
- ii. 118 ml M171
- iii. 118 ml DMEM/F12
- iv. 12.5 ml Fetal Bovine Serum (FBS) to reach a final concentration of 2.5% (v/v).

[00106] As explained above, DMEM/F12 medium is a 1:1 mixture of DMEM and Ham’s F12 medium. Thus, 118 ml DMEM/F12 medium contain 59 ml DMEM and 59 ml F12.

Accordingly, when using this method of making a culture medium, the final concentrations (v/v) with 500 ml total volume are as follows:

- DMEM: 250 ml + 59 ml = 309 ml, corresponds to $309/500 = 61.8\%$ (v/v)
- M171: 118 ml, corresponds to $118/500 = 23.6\%$ (v/v)
- F12: 59 ml, corresponds to $59/500 = 11.8\%$ (v/v).

[00107] Embodiments of this method of making a culture medium further comprise adding

v. 1 ml EGF stock solution (5 $\mu\text{g/ml}$) to achieve a final EGF concentration of 10ng/ml, and

vi. Insulin 0.175 ml stock solution (14.28 mg/ml) to achieve a final insulin concentration of 5 $\mu\text{g/ml}$.

[00108] It is noted here that in these embodiments, the above-mentioned volumes of these components i. to vi. will result in a final volume of 499.675 ml culture medium. If no further components are added to the culture medium, the remaining 0.325 ml (to add up to a volume of 500 ml) can, for example, be any of components i. to iv., that means either DMEM, M171, DMEM/F12 or FBS. Alternatively, the concentration of the stock solution of EGF or Insulin can of course be adjusted such that the total volume of the culture medium is 500 ml. In addition, it is also noted that components i. to iv. do not necessarily have to be added in the order in which they are listed but it is of course also possible to use any order to mix these components to arrive at the culture medium of the present invention. This means, that for example, M171 and DMEM/F12 can be mixed together and then combined with DMEM and FBS to reach final concentrations as described here, i.e. a final concentration of DMEM of about 55 to 65 % (v/v), a final concentration of F12 of about 5 to 15 % (v/v), a final concentration of M171 of about 15 to 30 % (v/v) and a final concentration of FBS of about 1 to 8 % (v/v).

[00109] In other embodiments, the method further comprises adding to DMEM a volume of 0.325 ml of one or more of the following supplements: adenine, hydrocortisone, 3,3',5-Triiodo-L-thyronine sodium salt (T3), thereby reaching a total volume of 500 ml culture medium. In this embodiment, the final concentration of these supplements in DMEM may be as follows:

about 0.05 to 0.1 $\mu\text{g/ml}$ adenine, for example about 0.025 $\mu\text{g/ml}$ adenine,
about 1 to 10 $\mu\text{g/ml}$ hydrocortisone,

about 0.5 to 5 ng/ml 3,3',5-Triiodo-L-thyronine sodium salt (T3), for example 1.36 ng/ml 3,3',5-Triiodo-L-thyronine sodium salt (T3).

[00110] In line with the above disclosure, a cell culture medium used herein is obtainable or that is obtained by the method of making the medium as described here.

[00111] In addition, a method of isolating mesenchymal stem cells from the amniotic membrane of the umbilical cord, wherein this method comprises cultivating amniotic membrane tissue in the culture medium prepared by the method is described here.

[00112] Thus, the present invention is also directed to (the use of) a cell culture medium comprising:

- DMEM in the final concentration of about 55 to 65 % (v/v),
- F12 in a final concentration of about 5 to 15 % (v/v),
- M171 in a final concentration of about 15 to 30 % (v/v) and
- FBS in a final concentration of about 1 to 8 % (v/v).

[00113] In certain embodiments of the culture medium described here, the medium comprises DMEM in the final concentration of about 57.5 to 62.5 % (v/v), F12 in a final concentration of about 7.5 to 12.5 % (v/v), M171 in a final concentration of about 17.5 to 25.0 % (v/v) and FBS in a final concentration of about 1.75 to 3.5 % (v/v). In other embodiments the culture medium may comprise DMEM in a final concentration of about 61.8 % (v/v), F12 in a final concentration of about 11.8 % (v/v), M171 in a final concentration of about 23.6 % (v/v) and FBS in a final concentration of about 2.5 % (v/v).

[00114] In addition, the culture medium may further comprise Epidermal Growth Factor (EGF) in a final concentration of about 1 ng/ml to about 20 ng/ml. In certain embodiments, the culture medium comprises EGF in a final concentration of about 10ng/ml. The culture medium described herein may further comprise Insulin in a final concentration of about 1 µg/ml to 10 µg/ml. In such embodiments the culture medium may comprise Insulin in a final concentration of about 5µg/ml.

[00115] The cell culture medium may further comprise at least one of the following supplements: adenine, hydrocortisone, and 3,3',5-Triiodo-L-thyronine sodium salt (T3). In certain embodiments the culture medium comprises all three of adenine, hydrocortisone, and 3,3',5-Triiodo-L-thyronine sodium salt (T3). If present, the culture medium may comprise adenine in a final concentration of about 0.01 to about 0.1 µg/ml adenine or of about 0.05 to about 0.1 µg/ml adenine, hydrocortisone in a final concentration of about 0.1 to about 10

$\mu\text{g/ml}$ hydrocortisone or of about 1 to about 10 $\mu\text{g/ml}$ hydrocortisone and/or 3,3',5-Triiodo-L-thyronine sodium salt (T3) in a final concentration of about 0.5 to about 5 ng/ml.

[00116] In embodiments of the cell culture medium, 500 ml of the cell culture medium of the present invention comprise:

- i. 250 ml of DMEM
- ii. 118 ml M171
- iii. 118 ml DMEM/F12
- iv. 12.5 ml Fetal Bovine Serum (FBS) (final concentration of 2.5%)

In further embodiments, the cell culture medium may further comprise

- v. EGF in a final concentration of 10ng/ml, and
- vi. Insulin in a final concentration of 5 $\mu\text{g/ml}$.

Both, insulin and and EGF can be added to to the culture medium using a stock solution of choice, such that the total volume of the culture medium does not exceed 500 ml.

[00117] In a particular example, the components i. to vi. of the culture medium used in the present invention are the components indicated in Figure 5, meaning they are obtained from the respective manufacturers using the catalogue number indicated in Figure 5. The medium that is obtained from mixing the components i. to vi. as indicated in Figure 5 is also referred herein as "PTT-6". It is again noted in this context that the constituents i. to vi. as well as any other ingredient such as an antibiotic of any other commercial supplier can be used in making the medium of the present invention.

[00118] In addition, the cell culture medium of the invention may comprise adenine in a final concentration of about 0.01 to about 0.1 $\mu\text{g/ml}$ adenine or of about 0.05 to about 0.1 $\mu\text{g/ml}$ adenine, hydrocortisone in a final concentration of about 0.1 to 10 $\mu\text{g/ml}$, of about 0.5 to about 10 $\mu\text{g/ml}$, or of about 1 to about 10 $\mu\text{g/ml}$ hydrocortisone and/or 3,3',5-Triiodo-L-thyronine sodium salt (T3) in a final concentration of about 0.1 to about 5 ng/ml or of about 0.5 to about 5 ng/ml.

[00119] To obtain the mesenchymal stem cell population as described herein the umbilical cord tissue may be cultured till a suitable number of (primary) mesenchymal cord lining stem cells have outgrown from the tissue. In typical embodiments, the umbilical cord tissue is cultivated until cell outgrowth of the mesenchymal stem cells of the amniotic membrane reaches about 70 to about 80% confluency. It is noted here that the term "confluency" or "confluence" is used in its regular meaning in the art of cell culture and is meant as an estimate/indicator of the number of adherent cells in a culture dish or a flask,

referring to the proportion of the surface which is covered by cells. For example, 50 percent confluence means roughly half of the surface is covered and there is still room for cells to grow. 100 percent confluence means the surface is completely covered by the cells, and no more room is left for the cells to grow as a monolayer.

[00120] Once a suitable number of primary cells (mesenchymal cord lining stem cells) have been obtained from the cord lining tissue by tissue explant, the mesenchymal stem cells are removed from the cultivation container used for the cultivation. By so doing, a master cell bank containing the (primary) isolated mesenchymal stem cells of the amniotic membrane can be obtained. Typically, since mesenchymal stem cells are adherent cells, removing is carried out using standard enzymatic treatment. For example, the enzymatic treatment may comprise trypsinization as described in International US patent application 2006/0078993, International patent application WO2006/019357 or International patent application WO2007/046775, meaning outgrowing cells can be harvested by trypsinization (0.125% trypsin/0.05% EDTA) for further expansion. If the harvested mesenchymal stem cells are, for example, used for generating a master cell bank, the cells can also be cryo-preserved and stored for further use as explained herein below.

[00121] Once being harvested, the mesenchymal stem cells can be transferred to a cultivation container for subculturing. The subculturing can also be started from frozen primary cells, i.e. from the master cell bank. For subculturing, any suitable amount of cells can be seeded in a cultivation container such as cell culture plate. The mesenchymal stem cells can, for this purpose, be suspended in a suitable medium (most conveniently, the culture medium PTT-6) for subculturing at a concentration of, for example, about 0.5×10^6 cells/ml to about 5.0×10^6 cells/ml. In one embodiment the cells are suspended for subcultivation at a concentration of about 1.0×10^6 cells/ml. The subculturing can be carried out by cultivation either in simple culture flasks but also, for example, in a multilayer system such as CellStacks (Corning, Corning, NY, USA) or Cellfactory (Nunc, part of Thermo Fisher Scientific Inc., Waltham, MA, USA) that can be stacked in incubators. Alternatively, the subculturing can also be carried out in a closed self-contained system such as a bioreactor. Different designs of bioreactors are known to the person skilled in the art, for example, parallel-plate, hollow-fiber, or micro-fluidic bioreactors. See, for example, Sensebe et al. "Production of mesenchymal stromal/stem cells according to good manufacturing practices: a review", *supra*. An illustrative example of a commercially available hollow-fiber bioreactor is the Quantum® Cell Expansion System (Terumo BCT, Inc). that has, for example, been

used for the expansion of bone marrow mesenchymal stem cells for clinical trials (cf., Hanley et al, Efficient Manufacturing of Therapeutic Mesenchymal Stromal Cells Using the Quantum Cell Expansion System, *Cytotherapy*. 2014 August ; 16(8): 1048–1058). Another example of commercially available bioreactors that can be used for the subculturing of the mesenchymal stem cell population of the present invention is the Xuri Cell Expansion System available from GE Healthcare. The cultivation of the mesenchymal stem cell population in an automated system such as the Quantum® Cell Expansion System is of particular benefit if a working cell bank for therapeutic application is to be produced under GMP conditions and a high number of cells is wanted.

[00122] The subculturing of the mesenchymal cord lining stem cells described herein takes place in a culture medium described herein such as the PTT-6 medium. Accordingly, the culture medium such as PTT-6 can be used both for the isolation of the mesenchymal stem cells from the amniotic membrane and the subsequent cultivation of the isolated primary cells by subcultivation. Also for the subcultivation, the mesenchymal stem cells can be cultured till a suitable number of cells have grown. In illustrative embodiments the mesenchymal stem cells are subcultured till the mesenchymal stem cells reach about 70 to about 80% confluency.

[00123] The isolation/cultivation of the population of mesenchymal cord lining stem cells can be carried out under standard conditions for the cultivation of mammalian cells. Typically, the method of the invention of isolating the population of the mesenchymal cord lining stem cells is typically carried out at conditions (temperature, atmosphere) that are normally used for cultivation of cells of the species of which the cells are derived. For example, human umbilical cord tissue and the mesenchymal cord lining stem cells, respectively, are usually cultivated at 37°C in air atmosphere with 5%CO₂. In this context, it is noted that the mesenchymal cells may be derived of any mammalian species, such as mouse, rat, guinea pig, rabbit, goat, horse, dog, cat, sheep, monkey or human, with mesenchymal stem cells of human origin being preferred in one embodiment.

[00124] Once a desired/suitable number of mesenchymal cord lining stem cells have been obtained from the subculture, the mesenchymal stem cells can be harvested by removing them from the cultivation container used for the subcultivation. The harvesting of the mesenchymal stem cells is typically again carried out by enzymatic treatment, including trypsination of the cells. The isolated mesenchymal stem cells are subsequently collected and are either directly used or preserved for further use. Typically, preserving is carried out by

cryo-preservation. The term “cryo-preservation” is used herein in its regular meaning to describe a process where the mesenchymal stem cells are preserved by cooling to low sub-zero temperatures, such as (typically) -80°C or -196°C (the boiling point of liquid nitrogen). Cryo-preservation can be carried out as known to the person skilled in the art and can include the use of cryo-protectors such as dimethylsulfoxide (DMSO) or glycerol, which slow down the formation of ice-crystals in the cells of the umbilical cord.

[00125] The isolated population of the mesenchymal cord lining stem cells that is obtained by the isolation method as described herein is highly defined and homogenous. In typical embodiments of the method at least about 90 % or more, about 91 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more of the isolated mesenchymal stem cells express the following markers: CD73, CD90 and CD105. In addition, in these embodiments at least about 90 % or more, about 91 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more of the isolated mesenchymal stem cells may lack expression of the following markers: CD34, CD45 and HLA-DR. In particular embodiments, about 97 % or more, about 98 % or more, or about 99 % or more of the isolated mesenchymal stem cell population express CD73, CD90 and CD105 while lacking expression of CD34, CD45 and HLA-DR.

[00126] Thus, in line with the above disclosure a mesenchymal stem population isolated from the amniotic membrane of the umbilical cord, wherein at least about 90 % or more cells of the stem cell population express each of the following markers: CD73, CD90 and CD105. In preferred embodiments at least about 91 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more cells of the isolated mesenchymal stem cell population are CD73+, CD90+ and CD105+, meaning that this percentage of the isolate cell population express each of CD73, CD90 and CD105 (cf. the Experimental Section of the present application) can be used herein. In addition, at least about 90 % or more, about 91 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more of the isolated mesenchymal stem cells may lack expression of the lack expression of the following markers. In particular embodiments about 97 % or more, about 98 % or more, or about 99 % or more cells of the isolated mesenchymal stem cell population express CD73, CD90 and

CD105 while lacking expressing of CD34, CD45 and HLA-DR. Such a highly homogenous population of mesenchymal stem cells derived from the amniotic membrane of the umbilical cord has been reported for the first time in US provisional application No. 62/404,582, filed October 5, 2016 as well as in co-pending US application Serial No. 15/725,913, filed 5 October 2017 as well as in co-pending PCT application PCT/SG2017/050500, also filed 5 October 2017, and meets the criteria for mesenchymal stem cells to be used for cellular therapy (also cf. the Experimental Section and, for example, Sensebe et al. "Production of mesenchymal stromal/stem cells according to good manufacturing practices: a review", supra). It is noted in this context that this mesenchymal stem cell population can be obtained by either the isolating method of the present invention but also by a different method such as cell sorting, if needed.

[00127] A method of making a culture medium for isolating mesenchymal stem cells as described herein can comprise, mixing to obtain a final volume of 500 ml culture medium:

- i. 250 ml of DMEM
- ii. 118 ml M171
- iii. 118 ml DMEM/F12
- iv. 12.5 ml Fetal Bovine Serum (FBS) to reach a final concentration of 2.5% (v/v).

[00128] As explained above, DMEM/F12 medium is a 1:1 mixture of DMEM and Ham's F12 medium.

[00129] Thus, 118 ml DMEM/F12 medium contain 59 ml DMEM and 59 ml F12. Accordingly, when using this method of making a culture medium, the final concentrations (v/v) with 500 ml total volume are as follows:

DMEM: $250 \text{ ml} + 59 \text{ ml} = 309 \text{ ml}$, corresponds to $309/500 = 61.8 \% \text{ (v/v)}$

M171: 118 ml, corresponds to $118/500 = 23.6 \% \text{ (v/v)}$

F12: 59 ml, corresponds to $59/500 = 11.8 \% \text{ (v/v)}$.

[00130] The present invention also relates to a method of treating a subject having a disease, the method comprising topically administering a mesenchymal stem cell population as described herein to the subject, wherein the mesenchymal stem cell population is administered within about 96 hours from the time point the mesenchymal stem cell population has been harvested.

[00131] Similarly, the present invention also relates to mesenchymal stem cell population as described herein for use in a method of treating a disease of a subject, wherein

the mesenchymal stem cell population is topically administered within about 96 hours from the time point the mesenchymal stem cell population has been harvested

[00132] The subject to be treated may be any suitable subject. The subject can be a vertebrate, more preferably a mammal. Mammals include, but are not limited to, farm animals, sport animals, pets, primates, dogs, horses, mice and rats. A mammal can also be a human, dog, cat, cow, pig, mouse, rat etc. Thus, in one embodiment, the subject is a vertebrate. The subject can also be a human subject. The subject therefore can be a subject in need of treatment. As such the subject may be afflicted with a disease as described elsewhere herein. In some embodiments the subject is afflicted with Type I or Type II diabetes with chronic foot ulcers. Preferably, the subject is negative for HLA antibodies to the mesenchymal stem cell population.

[00133] The mesenchymal stem cell population may be applied in any dosage. The dosage may be therapeutically effective. The “therapeutically effective amount/dosage” can vary with factors including but not limited to the activity of the cells used, stability of the cells in the patient's body, the severity of the conditions to be alleviated, the age and sensitivity of the patient to be treated, adverse events, and the like, as will be apparent to a skilled artisan. The amount of administration can be adjusted as the various factors change over time.

[00134] The dosage in which the mesenchymal stem cells are applied can also be a unit dosage. For example, the mesenchymal stem cell population can be applied in a unit dosage of about 20 million cells, of about 15 million cells, of about 10 million cells, of about 5 million cells, of about 4 million cells, of about 3 million cells, of about 2 million cells, of about 1 million cells, of about 0.5 million cells, of about 0.25 million cells or of less than 0.25 million cells. In a particular embodiment, the mesenchymal stem cell population is applied in a unit dosage of about 10 million cells.

[00135] The mesenchymal stem cells may be applied several times to the same subject. For example, stem cells are applied once, twice, three times or more a week. In principle any unit dosage of mesenchymal stem cells may be applied for the number of times suitable to cure or alleviate the disease. For example, the mesenchymal stem cell population can be applied once, twice three times or more a week. The mesenchymal stem cell population may also be applied for one, two, three, four, five, six, seven, eight, nine, ten, eleven weeks or more.

[00136] Thus, the unit dosage of about 20 million cells, of about 15 million cells, of about 10 million cells, of about 5 million cells, of about 4 million cells, of about 3 million cells, of about 2 million cells, of about 1 million cells, of about 0.5 million cells, of about 0.25 million cells or of less than 0.25 million cells is administered once or twice a week. The unit dosage of about 20 million cells, of about 15 million cells, of about 10 million cells, of about 5 million cells, of about 4 million cells, of about 3 million cells, of about 2 million cells, of about 1 million cells, of about 0.5 million cells, of about 0.25 million cells or of less than 0.25 million cells can also be administered one or twice a week for a period of time of three weeks, of four weeks, or five weeks or of six weeks, or of seven weeks, or of eight weeks or of ten weeks or more weeks.

[00137] It is also contemplated by the method of treatment of the present invention that the mesenchymal stem cell population is applied in a dosage of about 1000 cells/cm² to about 5 million cells/cm². Here, the expression cm² means the area of the wound/skin to which the stem cells are applied. It is also envisioned that the mesenchymal stem cell population is applied in a dosage of about 100,000 cells/cm², 300,000 cells/cm² or 500,000 cells/cm². The mesenchymal stem cell population can also be applied two times a week for about 8 weeks in a dosage of about 100,000 cells/cm², about 300,000 cells/cm² or about 500,000 cells/cm².

[00138] The mesenchymal stem cell population is administered within about 96 hours from the time point where the mesenchymal stem cell population has been harvested. How harvesting can take place is described elsewhere herein. It is also possible that the mesenchymal stem cell population is applied within about 72 hours, about 48 hours, about 24 hours, about 12 hours, about 6 hours or less from the time point where the mesenchymal stem cell population has been harvested. Between the time of harvesting and application, the mesenchymal stem cell population may be transported or stored by the method of transporting/storing of the present invention. Thus, aspects as described for the method of transporting/storing of the present application equally relate to the method of treatment of the present invention *mutatis mutandis*.

[00139] The method of treatment of the present invention serves to alleviate a disease suffered by the subject. In principle, any disease that may be treated by the mesenchymal stem cell population as described herein is meant here. In particular, the disease may be a skin disease or a wound. The wound may be caused by any cause e.g. by a burn, a bite, a trauma, a surgery, or a disease. The wound can also be caused by diabetic disease. Therefore, the wound can also be a diabetic wound. The wound may also be a diabetic foot ulcer.

Notably, the mesenchymal stem cell population may, for example, be placed directly onto a wound such as a burn or a diabetic wound (see International patent application WO2007/046775).

[00140] As described herein, between the harvesting of the mesenchymal stem cell population as described herein and their application to a subject the cells may be transported/stored in the carrier as defined herein. Therefore, the method of treating a subject of the present invention may also comprise the step of separating the mesenchymal stem cell population from the carrier before administering the mesenchymal stem cell population to the subject. The person skilled in the art knows how to perform the separation of cells from a carrier. For example, the separating of the mesenchymal stem cell population from the carrier may comprise centrifugation. Additionally or alternatively, separating the mesenchymal stem cell population from the carrier can comprise withdrawing the cell population from the vial by means of syringe.

[00141] After separating the stem cells from the carrier or after harvesting the mesenchymal stem cells or after obtaining mesenchymal stem cell population as described herein by any other method these cells are topically applied to a subject. In principle any way of topical administration is meant herein. The administering the mesenchymal stem cell population may be performed by means of a syringe. It is however also possible, to contact the mesenchymal stem cells within a cream, ointment, gel, suspension or any other suitable substance before applying the mesenchymal stem cells to the subject. The mesenchymal stem cell population after application to the subject may be held in place e.g. by a dressing such as Tegaderm® dressing and a crepe bandage to cover the Tegaderm® dressing. For a more even distribution of cells the application site may be gently massaged.

[00142] The present invention also relates to a unit dosage comprising about 20 million cells, of about 15 million cells, of about 10 million cells, of about 5 million cells, of about 4 million cells, of about 3 million cells, of about 2 million cells, of about 1 million cells, of about 0.5 million cells, of about 0.25 million cells or of less than 0.25 million cells of a mesenchymal stem cell population as described herein.

[00143] It is also envisioned that the unit dosage comprises about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2, about 1, about 0.5, about 0.25, or about 0.1 million cells. Preferably the unit dosage comprises about 10 million cells. It is further envisioned that the unit dosage comprises about 1000 cells to about 5 million cells. The unit dosage can be applied in a dosage of about 100,000 cells, 300,000 cells or 500,000 cells. As

described herein the unit dosage may be applied topically. For example, the unit dosage may be applied topically per cm².

[00144] The unit dosage can be applied once, twice, three times or more a week. For example, the unit dosage can be applied for one, two, three, four, five, six, seven, eight, nine, ten, eleven weeks or more. The unit dosage comprising of about 100,000 cells, about 300,000 cells or about 500,000 cells can be applied two times a week for 8 weeks, preferably onto 1 cm².

[00145] The unit dosage can be contained in any suitable container. For example, the unit dosage can be contained in a 1 ml vial. In such cases, for example 0.1 ml of the vial can be applied onto the subject, preferably per cm². The unit dosage may alternatively be contained in a syringe.

[00146] The unit dosage of the present invention the cells can be in contact with a liquid carrier as defined herein. If this is the case then the mesenchymal stem cells are separated from the carrier before administration. For example, the cells can be centrifuged and isolated before administration to a subject. The carrier may be any carrier as described herein, such as HypoThermosol™ or Hypothermosol™-FRS

[00147] The method of treatment and the unit dosage of the present invention can comprise utilization of viable cells. How viability can be tested is described elsewhere herein.

[00148] The invention will be further illustrated by the following non-limiting Experimental Examples.

[00149] Sequences as used herein are depicted in below Table 1.

[00150] Table 1. Sequences as used herein.

| SEQ ID NO. | What | Sequence |
|------------|------|----------|
| | | |

| SEQ ID NO. | What | Sequence |
|------------|---|---|
| 1 | CD73 identifier P21589 of Uniprot, version number 1 as of May 1, 1991: | MCPRAARAPATLLALGAVLWPAAGAWELTILHTNDVHSRLEQTSSEDS SKCVNASRCMGGVARLFTKVQQIRRAEPNVLLLDAGDQYQGTIWFTVY KGAEVAHF MNALRYDAMALGNHEFDNGVEGLIEPLLKEAKFPILSANIK AKGPLASQISGLYLPYKVLVPGDEVVGVIVGYTSKETPFLSNPGTNLVFED EITALQPEVDKLTNLV NKIIALGHSGFEMDKLIAQKVRGV D V V V G G H S NTFLYTG N P P S K E V P A G K Y P F I V T S D D G R K V P V V Q A Y A F G K Y L G Y L K I E FDERGNVISSHG N P I L L N S S I P E D P S I K A D I N K W R I K L D N Y S T Q E L G K T I V Y LDGSSQSCRFRECNMGNLICDAMINNL R H T D E M F W N H V S M C I L N G G G IRSPIDERNNGTITWENLAAVL P F G G T F D L V Q L K G S T L K K A F E H S V H R Y G QSTGEFLQVGGI H V V Y D L S R K P G D R V V K L D V L C T K C R V P S Y D P L K M D E VYKVL P N F L A N G G D G F Q M I K D E L L R H D S G D Q D I N V V S T Y I S K M K V I Y P AVEGRIKFSTGSHCHGSFSLIFLSLWAVIFVLYQ |
| 2 | CD90 identifier P04216 of Uniprot, version number 2 as of May 2, 2002: | MNLAISIALLLTVLQVSRGQKV T S L T A C L V D Q S L R L D C R H E N T S S S P I Q Y EFSLTRET K K H V L F G T V G V P E H T Y R S R T N F T S K Y N M K V L Y L S A F T S K D E GTYTCALHHS GH S P P I S S Q N V T V L R D K L V K C E G I S L L A Q N T S W L L L L L L S L S L L Q A T D F M S L |
| 3 | CD105 identifier P17813 of Uniprot, version number 2 as of July 15, 1998: | MDRGTLPLAV ALLASCSLSPTSLAETVHC DLQPVGPERGEV TYTTSQVS KGCVAQAPNAILEVHVL FLEFPTGPSQLELTLQASKQNGTWPREVLLVL SVNSSVFLHLQALGIPLHLAYNSSLVTFQEP PGVNTTELPSFPKTQILEWA AERGPITSAE LNDPQSILLRLGQAQGSLSFCM LEASQDMGRTLEWRPRT PALVRGCHLEGVAGHKEAHILRVLP GHSAGPRTVTVKVELSCAPGD L D A VLILQGP PYVSWLIDANHNMQIWT TGEYSFKIFPEKNIRGFKL P D T P Q G L LGEARMLNASIVASFVELPLASIVSLHASSCGGRLQTS PAPIQTTPPKDTC SPELLMSLIQTKCADDAMTLV LK K E L V A H L K C T I T G L T F W D P S C E A E D R GDKFVLRSA YSSCGMQVSASMISNEAVVNILSSSSPQRKKVHCLNMDSL SFQLGLYLSPHFLQASNTIEPGQSFVQVRVSPSVSEFLLQLDSCHLDLGP EGGTVELIQGRAAKGNCVSLLSPEGDPRFSFLLHFYTVPIPKTGTLSCT VALRPKTGSQDQEVHRTVFMRLNIISPDL S G C T S K G L V L P A V L G I T F G A F LIGALLTAALWYIYSHTRSPSKREP V V A V A A P A S S E S S T N H S I G S T Q S T P CSTSSMA |
| 4 | CD34 identifier P28906 of Uniprot, version number 2 as of July 15, 1998: | MLVRRGARAGPRM PRGWTALCLLSLLPSGFMSLDNNGTATPELPTQGT FSNVSTNVSYQETTPSTL GSTSLHPVSQH GNEATTNITETT VKFTSTSVIT SVYGNTNSSVQSQTSVISTVFTTPANVSTPETTLKPSLSPGNVSDLSTTSTS LATSPTKPYTSSSPILSDIKAEIKCSGIREV KLTQGICLEQNKTSSCAEFKK DRGEGLARVLCGEEQADADAGA QVCSLLLAQSEVRPQCLLLVLANRTEI SSKLQLMKKHQSDLK KLGILDFTEQDVASHQSYSQKTLIALVTSGALLA VLGITGYFLMNRRSWSPTGERLGEDPYTENG G G Q G Y S S G P G T S P E A Q G KASVNRGAQENGTGQATSRNGHSARQHVVADTEL |
| 5 | CD45 identifier P08575 of Uniprot, version number 2 as of July 19, 2003: | MYLWLKLLAFGFAFLDTEVFVTGQSPTPSPTGLTTAKMPSVPLSSDPLPT HTTAFSPASTFERENDFSETTSLSPDNTSTQVSPDSL DNASAFNTTG VSS VQTPHLP THADSQTPSAGTDTQTFSGSAANAKLNPTPGSNAISDVP GERS TASTFPTDPVSPLTTTSLAHSSAALPARTSNTTITANTSDAYLNASETT TLSPSGSAVISTTTIATTPSKPTCDEKYANITVDYLYNKETKLFTAKLNVN ENVECGNNTCTNNEVHNLTECKNASVSISHNSCTAPDKTLILDVPPGVEK FQLHDCTQVEKADTTICLKWKNIETFTCDTQNITYRFQCGNMIFDNKEIK |

| SEQ ID NO. | What | Sequence |
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| | | LENLEPEHEYKCDSEIL YNNHKFTNASKIIKTDGFGSPGEPQIIFCRSEAAHQ GVITWNPPQRSFHNFTLCYIKETEKDCLNLDKNLIK YDLQNLKPYTKYV LSLHAYIIAKVQRNGSAAMCHFTTKSAPPSQVWNMTVSMTSDNSMHVK CRPPDRNGPHERYHLEVEAGNTLVRNESHKNCDFRVKDLQYSTDYTF KAYFHNGDYPGEPFILHHSTSYNSKALIAFLAFLIIVTSIALLVLYKIYDL HKKRSCNLDEQQELVERDDEKQLMNVVEPIHADILLETYKRKIADEGRLF LAEFQSIPRVFSKFPKEARKPFNQNKNRYVDILPYDYNRVELSEINGDAG SNYINASYIDGFKEPRKYIAAQGPRDETVDDFWRMIWEQKATVIVMVTR CEEGNRNKCAEYWPSMEEGTRAFGDVVVKINQHKRCPDYIIQKLNIVNK KEKATGREVTHIQFTSWPDHGVPEDPHLLKLRRRVNAFSNFFSGPIVVH CSAGVGRTGT YIGIDAMLEGLEAENKVDVYGYVVKLRRQRCLMVQVE AQYILIHQALVEYNQFGETEVNLSLHPYLHNMKKRDPSPSEPLEAEFQ RLPSYRSWRTQHIGNQEENKSKNRNSNVIPYDYNRVPLKHELEMSKESE HDSESSDDSDSEEPSKYINASFIMSYWKPEVMIAAQGPLKETIGDFWQ MIFQRKVIVMLTELKHGDQEICAQYWGEKGQTYGDIEVDLKD TDKS STYTLRVFELRH SKRKDSRTVYQYQYTNWSVEQLPAEPKELISMIQVVK QKLPQKNSSEGNKHHKSTPLLIHCRDGSQQTGIFCALLNLLSAETEEVV DIFQVVKALRKARPGMVSTFEQYQFLYDVIASTYPAQNGQVKKNNHQE DKIEFDNEVDKVKQDANCVNPLGAPEKLPEAKEQAEGSEPTSGTEGPEH SVNGPASPALNQGS |
| 6 | HLA-DR identifier P01903 of Uniprot, version number 1 as of July 21, 1986: | MAISGVPVLGFFIIAVLMSAQESWAIKEEHVIIQAEFYLNPDQSGEFMDFD DGDEIFHVDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMT KRSNYTPITNVPPEVTVL TNSPVELREPNVLCIFDKFTPPVVNVTWLRNG KPVTGTGSETVFLPREDHLFRKFHYLPFLPSTEDVYDCRVEHWGLDEPLL KHWEFDAPSPLETTENVV CALGLTVGLVGIIGTIFIIGVRKSNA AERR GPL |
| 7 | Human TGFbeta1 Uniprot no: P36897 version number 1 as of June 1, 1994 | MEAAVAAPRPRLLLLVLA AAAAAAAAAALLPGATALQCFCHLCTKDNFTCV DGLCFVSVTETTDKVIHNSMCIAEIDLIPDRPFVCAPSSKTGSVTTTYCCN DHCNKIELPTTVKSSPGLGPVELAAVIAGPVCFVCISLMLMVYICHNRTVIE HRVPNEEDPSLDRPFISEGTTLKDLIYDMTTSGSGSGLPLL VQRTIARTIVLC ESIGKGRFGEVWRGKWRGEEVAVKIFSSREERSWFREA EIQYQVMLRHEN LGFIAADNKDNGTWTQLWLVS DYHEHGLFDYLNRYTVTVEGMIKLALS ASGLAHLHMEIVGTQGKPAIAHRDLKSKNILVKKNGTCC IADLGLAVRHD ATDTIDIAPNHRVGTKRYMAPEVLDD SINMKHFESFKRADIYAMGLV FWE ARRC SIGGIHEDYQLPYYDLVPSDPSVEEMRKVVCEQKLRPNIPNRWQSCE ALRVM AKIMRECWYANGAARLTALRIKKTLSQLSQEGIKM |
| 8 | Human VEGFA Uniprot no: P15692 version number 2 as of November 16, 2001 | MNFLLSWVHWSLALLLYLH HAKWSQAAPMAEGGGQNHHEVV KFM DVY QRSYCHPIETLVDIFQEY PDEIEYIFKPCSVPLMRCGGCCNDEGLECVPT EES NITMQIMRIKPHQGQHIGEMSFLQH NKCECRPKKDRARQEKKSVRGK GK QKRKRKKSRYKSWSVYVGARCC LMPWSLPGPHPCGPCSERRKHLFVQDP TCKCCKNTDSRCKARQLELNERTCRCDKPRR |
| 9 | HUMAN Platelet-derived growth factor receptor alpha | MGTSHPAFLVLGCLLTGLSLILCQLSLPSILPNENEKVVQLNSSFSLRCFGES EVSWQYPMSEEESSDVEIRNEENNSGLFVTVLEVSSASAAHTGLYTCYYNI TQTEENELEGRHIYIYVDPDVA FVPLGMTDYLVIVEDDDSAIIPCRTTDPE PVTLHNSEGVPASYDSRQGFNGTFTVGPYICEATVKGKKFQTIPFNYYAL |

| SEQ ID NO. | What | Sequence |
|------------|---|---|
| | Uniprot no: P16234, version number 1 as of April 1, 1990 | KATSELDLEMEALKTVYKSGETIVVTCVFNNEVVLDLQWTPGGEVKGKG TMLEEIKVPSIKLVYTLTVPEATVKDSGDYECARQATREVKEMKKVTISV HEKGFIEIKPTFSQLEAVNLHEVKHFVVEVRAVPPPRISWLKNNLTIENLT ITTDVEKIQEIRYRSKLLIRAKEEDSGHYTIVAQNEDAVKSYTFELLTQVP SILDLVDDHHGSTGGQTVRCTAEGTPLPDIEWMICKDIKCNNETSWTILA NVSNIITEIHSRDRSTVEGRVTFKVEETIAVRCLAKNLLGAENRELKLVAF LRSELTVA AAVLVLLVIVIIISLIVLVVIWKQKPRYEIRWRVIESISPDGHEYY VDPMQLPYDSRWEFPRDGLVLGRVLGSGAFGKVVVEGTAYGLSRSQPVMK VAVKMLKPTARSSEKQALMSELKIMTHLGPLNIVNLLGACTKSGPIYIITE YCFYGDLVNYLHKNRDSFLSHHPEKPKKELDIFGLNPADESTRSYVILSFEN NGDYMDMKQADTTQYVPMLEKVEVSKYSDIQRSLYDRPASYK KKSMLDS EVKNLLSDDNSEGLTLLDLSFTYQVARGMEFLASKNCVHRDLAARNVLL AQQKIVKICDFGLARDIMHDSNYVSKGSTFLPVKWMAPESIFDNLTTLS VWSYGILLWEIFSLGGTPYPGMMVDSTFYNKIKSGYRMAKPDHATSEVYE MVKCWNSEPEKRPSFYHLSEIVENLLPGQYKKSYEKIHLDFLKS DHPVA MRVDSDNA YIGVTYKNEEDKLDWEGGLDEQRLSADSGYIPLPDIDPVPE EEDLGKRRRHSSQTSEESA IETGSSSSTFIKREDETIEDIDMMDDIGIDSSDL EDSFL |
| 10 | Human Ang-1 Uniprot no: Q15389 version number 2 as of January 1, 1998 | MTVFLSFAFLAAILTHIGCSNQRRSPENSGRRYNRIQHGCAYTFILPEHD GNCRESTTDQYNTNALQRDAPHVEPDFSSQKLQHLEHVMENYTQWLQ KLENYIVENMKSEMAQIQNAVQNHTATMLEIGTSLLSQTAEQTRKLT VETQVLNQTSRLEIQLENSLSTYKLEKQLLQQTNEILKIHEKNSLLEHKI LEMEGKHKEELDTLKEEKENLQGLVTRQTYIIQELEKQLNRATTNNSVL QKQQLELMDTVHNLVNLCTKEGVLLKGGKREEEKPFPRDCADVYQAGF NKSIGYTIYINNMPEPKKVFCNMDVNGGGWTVIQHREDGSLDFQRGWK EYKMGFGNPSGEYWLGNFIFAITSQRQYMLRIELMDWEGNRAYSQYD RFHIGNEKQNYRLYLKGTGTAGKQSSLILHGADFSTKADNDNCMCK CALMLTGGWWFDACGPSNLNGMFYTAGQNHGKLNGLIKWHYFKGPSYS LRSTTMMIRPLDF |
| 11 | Human HGF Uniprot no: P14210 version number 2 as of August 1, 1991 | MWVTKLLPALLLQHVLHLHLLLPPIAIPYAEGQRKRNTIHEFKSAKTTL IKIDPALKIKTKKVNTADQCANRCTRNKGLPFTCKAFVFDKARKQCLWF PFNSMSSGVKKEFGHEFDLYENKDYIRNCIIGKGRSYKGTVSITKSGIKCQ PWSSMIPHEHSFLPSSYRGKDLQENYCRNPRGEEGGPWCFSTNPEVRYE VCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTPHRHKFLPE RYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMND TDVPLETTECIQGQGEYRGTVNTIWNIGIPCQRWDSQYPHEHDMTPENF KCKDLRENYCRNPDGSESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYR GNGKNYMGNLSQTRSGLTCSMWDKNMEDLHRHIFWEPDASKLNENYC RNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNLDHPVISCATK KQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCPSR DLKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPESDLVLMKLAR PAVLDDFVSTIDLPNYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLIYI MGNEKCSQHHRGKVTLNESEICAGA EKIGSGPCEGDYGGPLVCEQHKM RMVLGVIVPGRGCAIPNRP GIFVRVAYYAKWIHKIILTYKVPQS |
| 12 | PDGFB human Uniprot no: P01127 version number 1 as of | MNRWALFSLCCYLRLVSAEGDPIPEELYEMLSDHSIRSFDLQRLHGHG DPGEEDGAELDLNMTRSHSGGELES LARGRSLGSLTIAEPAMIAECKTR TEVFEISRRLIDRTNANFLVWPPCVEVQRCSGCCNNRNVQCRPTQVQLR PVQVRKIEIVRKKPIFKKATVTLEDHLACKCETVAAARPVTRSPGGSQE RAKTPQTRVTIRTVRVRRPPKGGKHRKFKHTHDKTALKETLGA |

| SEQ ID NO. | What | Sequence |
|------------|---|---|
| | July 21, 1986 | |
| 13 | Human IL-10 Uniprot no: P22301 version number 1 as of August 1, 1991 | MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDLRDAFS RVKTFFQMKDQLDNLLKESLLEDFKGYLGQCQALSEMIQFYLEEVMPPQ AENQDPDIKAHVNSLGENLKTLLRRLRRCHRFLPCENKSKAVEQVKNAF NKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN |

Experimental Examples

[00151] 1. Cryopreservation of Umbilical Cord Tissue Prior to Isolation of Mesenchymal Stem Cells

[00152] Umbilical cord tissue (the umbilical cords were donated with informed consent of the mother) was processed for the subsequent isolation of the mesenchymal stem cells from the amniotic membrane of the umbilical cord as follows.

[00153] 1.1 Washing of umbilical cord tissue sample:

- a. Remove scalpels from the protective cover.
- b. Hold the umbilical cord securely using the forceps and cut the cord into a 10 cm length piece using a scalpel. Place the unused cord back in the original tissue cup.
- c. Transfer the 10 cm long umbilical cord piece into a new 150 mm culture dish. The 150mm culture dish may be used in place of the cups.
- d. Use the cover of the 150 mm culture dish as a resting place for forceps and scalpel.
- e. Remove 25ml Plasmalyte A (Baxter, Catalog # 2B2543Q) with a 30 ml syringe. Hold the syringe at a 45° angle using one hand and dispense the Plasmalyte A directly onto the umbilical cord tissue.
- f. Holding the culture dish at a slight angle remove the Plasmalyte A with a 30 ml syringe and blunt needle.
- g. Collect used Plasmalyte A in a 300 ml transfer bag that serves as a trash container and dispose it in the biohazard bin.
- h. Repeat wash procedure, if necessary using a new culture dish for each wash. Make sure all blood clots on the surface have been removed. More Plasmalyte A can be used if needed to clean the tissue.

- i. Place the tissue into a new labeled tissue culture dish to continue cutting the tissue. Place 20 ml of Plasmalyte A into the dish so the tissue does not dry out while cutting it.
- j. Cut the cords into equal approximately 1-cm sections resulting in 10 sections in total.
- k. Further cut each 1 cm section into smaller pieces with approximately 0.3 cm x 0.3 cm to 0.5 cm x 0.5 cm per section.
- l. Remove any Plasmalyte A that is in the dish.
- m. Pull 25 ml Plasmalyte A with a 30 ml syringe from the original Plasmalyte A bag and dispense directly on the umbilical cord tissue pieces.
- n. Hold culture dish in an angle to collect all Plasmalyte A used for washing the tissue on one side and remove it with a syringe and blunt needle.
- o. Repeat wash one more time. There should not be any clots left.

[00154] NOTE: If the cord is not frozen right away, the umbilical cord tissue is kept in Plasmalyte A until ready to freeze.

[00155] **1.2 Cryopreservation of umbilical cord tissue:**

- a. Prepare cryopreservation solution:
 - i. Prepare 50 ml freezing solution consisting of 60% Plasmalyte A, 30% of 5% Human Serum Albumin, and 10% dimethyl sulfoxide (DMSO).
 - ii. Label a 150 ml transfer bag with “Tissue freeze solution” and attach a plasma transfer set to the port using aseptic technique.
 - iii. Remove 30 ml Plasmalyte A with a 30 ml Syringe from the original Plasmalyte A bag and transfer it in the transfer bag labeled “tissue freeze solution” with the time and date solution is made.
 - iv. Remove 15 ml of 5% Human Serum Albumin with a 20 ml syringe and transfer it into the labeled transfer bag.
 - v. Add 5 ml DMSO to the transfer bag.
 - vi. Mix well and record mixing of freeze solution
- b. Remove the Plasmalyte A from the tissue before adding the freeze solution.
- c. Using a 60 ml syringe, pull all 50 mls of the freeze solution into the syringe add approximately 30 ml freeze solution to the 150 mm cell culture dish containing the umbilical cord tissue. Place a blunt needle on the syringe to keep it sterile.
- d. Swirl the culture dish containing the tissue and freezing solution every minute for 10 minutes.

- e. Using forceps, select 8 randomly chosen sections and place them in each of the four 4 ml cryovials. Select 4 randomly chosen sections and place them into one 1.8 ml cryovial. These sections should be free of blood clots.
- f. Fill each cryovial containing the umbilical cord tissue with the remaining freezing solution to the 3.6 ml filling line for the 4 ml tubes and the 1.8 ml line for the 1.8 ml Nunc vial.
- g. Label one Bactec Lytic/10 - Anaerobic/F and one Bactec Plus Aerobic/F bottle with tissue ID.
- h. Remove 20 ml freeze solution from the culture dish with a syringe and a blunt needle, after wiping the Bactec vials with an alcohol swab, switch the blunt needle for an 18g needle and inoculate the aerobic and the anaerobic Bactec bottles with 10 ml each.
- i. Start controlled rate freezer.
- j. After controlled rate freeze is completed place the units in a continuous temperature monitored liquid nitrogen freezer until further use.

[00156] 2. Isolation of Mesenchymal Cord Lining Stem Cells from umbilical cord tissue

[00157] 2.1. Preparing media for processing MSCs from umbilical cord tissue:

- a. To make 500 ml PTT6 (culture/growth media) add the following in the order listed:
 - i. DMEM, 250 ml
 - ii. M171 118 ml
 - iii. DMEM F12 118 ml
 - iv. FBS 12.5 ml (final concentration of 2.5%)
 - v. EGF 1 ml (final concentration of 10ng/ml)
 - vi. Insulin 0.175 ml (final concentration of 5 μ g/ml)

[00158] The above-mentioned volumes of components i. to vi when result in a final volume of 499.675 ml culture medium. If no further components are added to the culture medium, the remaining 0.325 ml (to add up to a volume of 500 ml) can, for example, be any of components i. to iv, that means either DMEM, M171, DMEM/F12 or FBS. Alternatively, the concentration of the stock solution of EGF or Insulin can of course be adjusted such that the total volume of the culture medium is 500 ml. Alternatively, a stock solution of an antibiotic such as Penicillin-Streptomycin-Amphotericin can be added to result in a final volume of 500 ml. It is also possible to add to the culture medium a volume of 0.325 ml of one or more of the following supplements: adenine, hydrocortisone, 3,3',5-Triiodo-L-thyronine sodium salt (T3), thereby reaching a total volume of 500 ml culture medium.

- vii. Label the bottle “PTT6” with date media was prepared, initial of the operator, and the phrase “expires on” followed by the expiration date. Expiration date is the earliest expiration date of any of the component or 1 month from the preparation date, whichever comes first.
- b. To make the rinse media (Hank’s Buffered Salt Solution (HBSS) without Calcium or Magnesium and with 5% FBS), add 2.5 ml FBS to 47.5 ml of HBSS in a 50 ml centrifuge tube. Label the tube “Rinse Media” with operator initials and date the media is made.
- c. All media will be tested for sterility using Bactec Lytic/10 – Anaerobic/F (Becton Dickinson & Company) and Bactec Pluc + Aerobic/F (Becton Dickinson & Company). Inject 20 ml of prepared media into each bottle.

[00159] 2.2 Thawing of umbilical cord tissue for MSC harvesting:

- a. Initiate the thaw once an operator is prepared to process the sample in the clean room. Do not thaw more than 1 vial at a time unless the vials originate from the same donor.
- b. Wipe the water bath with disinfectant followed by 70% isopropanol and fill it with 1 L sterile water. Heat the water bath up to 36-38°C.
- c. Prepare 10 mL of rinse medium consisting of 70% to 90% PlasmaLyte A in the clean room under a biosafety cabinet. Sterile filter the solution with a 0.2-µm syringe filter attached to a 10 ml syringe and keep the solution refrigerated until use.
- d. Place a processing label on a 50 ml conical tube.
- e. Confirm water bath temperature is at 36-38°C.
- f. Take vial(s) of tissue from the liquid nitrogen storage and thaw rapidly in the 37°C water bath filled with 1L of sterile water. The vial holder for the Mr. Frosty Nalgene Cryo 1°C freezing container floats with vials in place and can be used as a floating rack when thawing samples.
- g. Remove the vial from the water bath and spray them with 70% Isopropanol solution. A good time to pull the vial from the water bath is when small ice can be seen floating in the vial – suggest internal temperature of the vial is less than 37°C.
- h. Place vial into pass-through and alert the clean room processing technician.

[00160] 2.3 Preparing for tissue processing:

- a. Umbilical cord tissue processing should be performed in an environmentally monitored (EM) clean room: At the end of each shift, full room and hood cleaning are performed
- b. Prepare/clean the biosafety cabinet.
- c. Perform viable particle counting while working in the biosafety cabinet.

- d. Assemble all necessary supplies in the biosafety cabinet checking each for packaging damage and expiration dates. When handling syringes, serological pipets, sterile forceps, scalpels, tissue plates, and needles, make sure not to touch any surface that will come in contact with the sterile product. Only the exterior of the syringe barrel, tubing, plunger tip and/or needle cap or sheath may be safely handled. Discard supply if the surface has been touched or has touched a non-sterile surface.
- e. Record lot numbers and expiration dates (if applicable) of all reagents and supplies to be used.
- f. Receive the thawed vial by cleaning the vial with lint-free wipe moistened with 70% alcohol before transferring into the biosafety cabinet.
- g. Using an aspirating needle with a syringe, withdraw as much liquid from the vial. Avoid suctioning the tissue.
- h. Using sterile forceps, transfer the tissue into a sterile 100 mm petri dish.
- i. Add an aliquot of 5 ml rinse medium to the tissue fragments.
- j. Swirl the contents for 15-30 seconds, then remove the rinse medium with a pipette or syringe with aspirating needle. Repeat this rinse process twice.
- k. Add 2 mL of rinse medium to the tissue to avoid drying out the tissue.

[00161] 2.4. Initiating MSC outgrowth from tissue:

- a. Label the bottom of a 6-well plate "Outgrowth 1" with MSC lot number or umbilical cord tissue ID and the date outgrowth is initiated. If 60 mm tissue culture dish is used, divide the plate into 4 quadrants by drawing a grid on the bottom of the dish.
- b. Using sterile, disposable forceps, place one 3 x 3 mm to 5 x 5 mm tissue into each well. If using a 60 mm tissue culture dish, place the tissue into the middle of each quadrant to keep the tissues apart (more than 1 cm from each other).
- c. Fill each well with 3 ml of PTT6.
- d. Using an aspirating needle coupled to 30 ml syringe, withdraw enough media to barely cover the tissue. Do not tilt the plate. Do not touch the bottom of the well with the aspirating needle.
- e. Using an inverted light microscope, observe for cell outgrowth every day (24 ± 6 hrs). Real time cell culture imaging system may be used in place of the light microscope.
- f. Change media every day. Be sure to equilibrate the media to room temperature before use.
- i. Aspirate off the medium.
- ii. Add 3 ml of PTT6.

- iii. Aspirate until tissue is barely submerged in the medium.
- g. When cellular outgrowth is observed from the tissue, transplant the tissue to a new 6-well plate using the same procedure as 4.a to 4.e above except label the plate “Outgrowth 2”. Maintain cell outgrowth in “Outgrowth 1” plate by adding 2ml of PTT6 to each well. Observe for confluency every day. Replace media every 2-3 days (be sure to equilibrate the media to room temperature before use).
- h. When cell outgrowth is observed in “Outgrowth 2” plate, repeat step 4.a to 4.e except label the plate “Outgrowth 3.” Maintain cell outgrowth in “Outgrowth 2” plate by adding 2 ml of PTT6 to each well. Observe for confluency every day. Replace media every 2-3 days (be sure to equilibrate the media to room temperature before use).
- i. When outgrowth is observed in “Outgrowth 3” plate, discard the tissue. If the tissues are very small and do not seem to interfere with cell growth, dispose of the tissue when subculturing.
- j. When cells reach 40-50% confluency, observe cells every days to prevent over-expansion.
- k. When cells reach 70-80% confluency, subculture the cells. Do not allow cells to expand beyond 80% confluence.

[00162] With the size of the tissue explants being about 1-3mm, and the tissue explant/cell culture is performed in 175 mm squared culture dishes, the average number of mesenchymal stem cells harvested from an explant is typically about 4,000 - 6,000 cells/explant. Accordingly, when the mesenchymal stem cells are simultaneously grown out of 48 explants about 300,000 cells can be obtained at harvest. These 300,000 mesenchymal stem cells collected from explants can then be used for subculturing by seeding a 175cm² cell culture flask with such 300,000 cells as described in the following Example 2.5 (this can be referred to as Passage 1). The mesenchymal stem cells obtained from this passage 1 can then be used to seed again 175cm² flasks (Passage 2) and expand the cells as described in the following Example 2.5. The cells obtained from both Passage 1 and Passage 2 can be “banked” by cryo-preservation, with the mesenchymal stem cells obtained after Passage 2 being considered to represent the Master Cell Bank which will be for further expansion of the mesenchymal stem cells, for example, in a bioreactor as explained below in Example 2.7.

[00163] **2.5. Subculturing MSC in cell culture dishes**

- a. Perform viable particle while working in the biosafety cabinet. Equilibrate all media to room temperature before use.
- b. When cell outgrowth reaches about 70-80% confluency, subculture cells.

- i. Remove PTT6 from the petri dish.
 - ii. Rinse with HBSS without Calcium or Magnesium.
 - iii. Add 0.2 ml 1X TrypLE-EDTA and swirl for 1-2 minutes.
 - iv. Tilt the dish 30-45° to allow cells to shift down by gravitational flow. Gentle tapping on the side of the plate expedites detachment.
 - v. Add 1ml of PTT6. Pipette up and down gently then transfer cells to a 15 ml centrifuge tube. Use clean pipette tip with each well. Cells from all 6 wells can be pooled into a single 15ml tube.
 - vi. Centrifuge for 10 minutes at 1200 rpm.
 - vii. Remove supernatant and resuspend cells with 5ml PTT6.
- c. Subculturing MSC
- i. Aliquot 50 μ l of the cell suspension and assay for TNC and viability by Trypan Blue Exclusion Assay.
 - ii. Count cells using a hemocytometer. Expect to count 20-100 cells/square. If the count higher than 100, dilute the original sample 1:5 and repeat Trypan Blue method using a hemocytometer.
 - iii. Calculate viable cells/ml and total viable cells:
 1. Viable cells/ml = viable cell count x dilution factor x 10^4
 2. Total viable cells = viable cell count x dilution factor x total volume x 10^4
 - iv. Calculate % viability:
 1. % viability = viable cell count x 100 / (viable cell count + dead cell count)
 - v. Dilute the cell suspension to 1.0×10^6 cells/ml:
 1. "X" volume = Total viable cells/ 10^6 cells/ml
 2. For example, if total viable cell number is 1.0×10^7 ;
 3. "X" = $10^7/10^6$ cells/ml or 10 ml, therefore, you would bring your total cell volume up to 10 ml by adding 5 ml to your cell suspension (that is at 5 ml).
 - vi. If the cell suspension is less than 10^6 /ml, determine the volume required to seed 2×10^6 cells for each 150 mm petri dish or 175 cm² flask.
 1. Volume for 2×10^6 cells = 2×10^6 cells \div viable cells/ml
 2. For example, if viable cells/ml is 8×10^5 cells/ml, 2×10^6 cells \div 8×10^5 cells/ml or 2.5 ml are needed.
 - vii. Set aside 0.5 ml for MSC marker analysis.
 - viii. Seed 2×10^6 cells to each 150 mm petri dish or 175 cm² flask with 30 ml PTT6.

ix. Observe cells for attachment, colony formation, and confluence every three days. When cells reach 40-50% confluence, observe cells every one-two days to prevent over-expansion. DO NOT allow cells to expand beyond 80% confluence. A real time cell culturing monitoring system can be used in place of the light microscope.

x. Replace media every 2-3 days.

[00164] 2.6 Cryopreserving MSC cells

a. Perform viable particle while working in the biosafety cabinet.

b. When cells reach 70-80% confluence, detach cells using 2 ml 1X TrypLE-EDTA for each 150 mm petri dish or 175 cm² flask.

i. Remove PTT6 from the petri dish.

ii. Wash with 5ml HBSS or PBS without calcium or magnesium.

iii. Add 2 ml 1X TrypLE-EDTA and swirl for 1-2 minutes.

iv. Tilt the dish 30-45° to allow cells to shift down by gravitational flow. Gentle tapping on the side of the petri dish helps to expedite detachment.

v. Add 10 ml PTT6 to inactivate TrypLE. Mix well to dissociate cell clumps.

vi. Transfer cells to 15 ml centrifuge tube using a Pasteur pipette.

vii. Centrifuge for 10 minutes at 1200 rpm.

viii. Aspirate medium and resuspend with 10 ml PTT6.

ix. Aliquot 50 µl and determine total viable cell number and % viability as above. Cell count will need to be performed within 15 minutes as the cells may start clumping.

c. Preparing cells for cryopreservation

i. Prepare Cell Suspension Media and Cryopreservation Media and freeze the cells

[00165] 2.7. Subculturing (expansion) of MSC in a Quantum Bioreactor (Terumo BTC, Inc.)

It is also possible to use a Quantum Bioreactor can used to expand the MSC. The starting cell number for the expansion in the Quantum Bioreactor should range between 20 to 30 million cells per run. The typical yield per run is 300 to 700 million MSC at harvest. The Bioreactor is operated following the protocol of the manufacturer. The so obtained mesenchymal stem cells are typically cryo-preserved (see below) and serve as Working Cell Bank.

[00166] MATERIALS/ REAGENTS:

1. Quantum Expansion Set
2. Quantum Waste Bag
3. Quantum Media Bag

4. Quantum Inlet Bag
5. PTT6
6. PBS
7. Fibronectin
8. TrypLE
9. 3ml syringe
10. Glucose test strips
11. Lactate test strips
12. 60ml Cell Culture Plate or equivalent
13. Medical Grade 5% CO₂ Gas-mix
14. 50ml Combi-tip

[00167] EQUIPMENT:

1. Biosafety Cabinet
2. Glucose Meter (Bayer Healthcare/Ascensia Contour Blood Glucose Meter)
3. Lactate Plus (Nova Biomedical)
4. Peristaltic pump with head
5. Centrifuge, Eppendorf 5810
6. Sterile Tube Connector
7. M4 Repeat Pipettor
8. RF Sealer

[00168] PROCEDURE:

1. Preparing the Quantum Bioreactor

- a) Priming the Quantum Bioreactor
- b) Coating the bioreactor:
 - 1) Prepare the fibronectin solution in the biosafety cabinet.
 - 1) Allow lyophilized fibronectin to acclimate to room temperature (≥ 15 min at room temperature)
 - 2) Add 5ml of sterile distilled water; do not swirl or agitate
 - 3) Allow fibronectin to go into solution for 30 min.
 - 4) Using a 10 ml syringe attached with an 18g needle, transfer the fibronectin solution to a cell inlet bag containing 95ml of PBS.
 - 2) Attach the bag to the “reagent” line

- 3) Check for bubbles (bubbles may be removed by using “Remove IC Air” or “Remove EC Air” and using “Wash” as the inlet source.
 - 4) Open or set up program for coating the bioreactor (Figure 1. Steps 3-5).
 - 5) Run the program
 - 6) While the program is running to coat the bioreactor, prepare a media bag with 4L of PTT6 media.
 - 7) Attach the media bag to the IC Media line using a sterile tube connector.
 - 8) When the bioreactor coating steps are completed, detach the cell inlet bag used for fibronectin solution using a RF sealer.
- c) Washing off excess fibronectin
 - d) Conditioning the bioreactor with media

2. Culturing the cells in the Quantum Bioreactor

- a) Loading and attaching the cells with Uniform Suspension:
- b) Feeding and cultivation of the cells
 - 1) Chose media flow rate to feed the cells.
 - 2) Sample for lactate and glucose every day.
 - 3) Adjust the flow rate of the media as the lactate levels increase. The actual maximal tolerable lactate concentration will be defined by a flask culture from which the cells originate. Determine if adequate PTT6 media is in the media bag. If necessary, replace the PTT6 media bag with a fresh PTT6 media bag.
 - 4) When the flow rate has reached the desired value, measure lactate level every 8-12 hours. If the lactate level does not decrease or if the lactate level continues to increase, harvest the cells.

3. Harvesting the cells from the Quantum Bioreactor

- a) When lactate concentration does not decrease, harvest the cells after sampling for lactate and glucose for the last time.
- b) Harvesting the cells:
 - 1) Attach cell inlet bag filled with 100ml TrypLE to the “Reagent” line using a sterile tube connector.
 - 2) Confirm sufficient PBS is in the PBS bag. If not, attach a new bag with at least 1.7 liters of PBS to the “Wash” line using a sterile tube connector.
 - 3) Run the Harvest program

4. Cryopreserving the cells

- 1) Once the cells have been harvested, transfer the cells to 50ml centrifuge tube to pellet the cells.
- 2) Resuspend using 25ml of cold cell suspension solution. Count the cells using Sysmex or Biorad Cell counter. Attach the cell count report to the respective Quantum Processing Batch Record.
- 3) Adjust cell concentration to 2×10^7 /ml
- 4) Add equal volume cryopreservation solution and mix well (do not shake or vortex)
- 5) Using a repeat pipettor, add 1ml of the cell suspension in cryopreservative to each 1.8ml vial. Cryopreserve using the CRF program as described in the SOP D6.100 CB Cryopreservation Using Controlled Rate Freezers
- 6) Store the vials in a designated liquid nitrogen storage space.
- 7) Attach the CRF run report to the form respective MSC P3-Quantum Processing Batch Record.

[00167] 3. Analysis of Stem Cell Marker Expression in Mesenchymal Cord Lining Stem Populations isolated from umbilical cord tissue, using different culture media

Flow cytometry experiments were carried out to analyse mesenchymal stem cells isolated from the umbilical cord for the expression of the mesenchymal stem cell markers CD73, CD90 and CD105.

[00168] For these experiments, mesenchymal stem cells were isolated from umbilical cord tissue by cultivation of the umbilical cord tissue in three different cultivation media, followed by subculturing of the mesenchymal stem cells in the respective medium as set forth in Example 2.

[00169] The three following culture media were used in these experiments: a) 90% (v/v) DMEM supplemented with 10 % FBS (v/v), b) the culture medium PTT-4 described in US patent application US 2008/0248005 and the corresponding International patent application WO2007/046775 that consist of 90% (v/v) CMRL1066, and 10% (v/v) FBS (see paragraph [0183] of WO2007/046775) and c) the culture medium of the present invention PTT-6 the composition of which is described herein. In this flow cytometry analysis, two different samples of the cord lining mesenchymal stem cell (CLMC) population were analysed for each of the three used culture media.

[00170] The following protocol was used for the flow cytometry analysis.

[00171] Materials and methods

| Instruments name | Company Name | Serial Name |
|---------------------------------|--------------|------------------|
| BD FACS CANDO | BD | V07300367 |
| Inverted Microscope, CKX41SF | Olympus | 4K40846 |
| Centrifuge, Micro spin Tabletop | Biosan | 010213-1201-0003 |

| Reagent list | Company Name | CatLog Number |
|--------------------|---------------|---------------|
| 10 X Trypsin | Biowest | X0930-100 |
| 10 X PBS | Lonza | 17-517Q |
| DMEM | Lonza | 12-604F |
| Fetal Bovine Serum | GE healthcare | A11-151 |

| Antibody list | Company Name | CatLog Number |
|---|--------------|---------------|
| Human CD73 Purified AD2 0.1mg | BD | 550256 |
| Human CD90 Purified 5E10 1mL | BD | 550402 |
| Human CD105 Purified 266 0.1mg | BD | 555690 |
| Alexa Fluor 647 goat anti-mouse IgG (H+L) *2 mg/mL* | BD | A21235 |

| Reagents name | Composition |
|---------------|--------------------------------------|
| 1 X PBS (1L) | 100ml of 10 X PBS + 900ml of sterile |

| | |
|---------------|---------------------------------|
| | distilled H2O |
| 1x PBA (50ml) | 49.5ml of 1XPBS + 0.5 ml of FBS |

[00172] Procedure

a) Cell isolation and cultivation from the umbilical cord lining membrane

1. Explant tissue samples were incubated in a cell culture plate and submerged in the respective medium, then keep it in CO₂ incubator at 37°C as explained in Example 2.
2. The medium was changed every 3 days.
3. Cell outgrowth from tissue culture explants was monitored under light microscopy.
4. At a confluence of about 70%, cells were separated from dish by trypsinization (0.0125% trypsin/0.05% EDTA) and used for flow cytometry experiments.

b) Trypsinization of cells for experiments

1. Remove medium from cell culture plate
2. Gently rinse with sterile 1X PBS to remove traces of FBS as FBS will interfere with the enzymatic action of trypsin.
3. Add 1X trypsin to cell culture plate and incubate for 3-5 min in 37 °C.
4. Observe cells under microscope to ensure that they are dislodged. Neutralize trypsin by adding complete media containing FBS (DMEM with 10% FBS).
5. Use a pipette to break up cell clumps by pipetting cells in media against a wall of the plate. Collect and transfer cell suspension into 50 ml centrifuge tubes
6. Add sterile 1X PBS to plate and rinse it, Collect cell suspension into the same centrifuge tube.
7. Centrifuge it at 1800 rpm for 10 mins.
8. Discard supernatant and re-suspend cell pellet with PBA medium.

c) Counting cells

1. Ensure that the haemocytometer and its cover slip are clean and dry, preferably by washing them with 70% ethanol and letting them dry before wiping them with Kim wipes (lint-free paper).
2. Aliquot a small amount of cells in suspension into a micro centrifuge tube and remove from the BSC hood.

3. Stain cells in suspension with an equal volume of Trypan Blue, e.g. to 500µl of suspension add 500µl of Trypan Blue (dilution factor = 2X, resulting in 0.2% Trypan Blue solution).
4. Avoid exposure of cells to Trypan Blue for longer than 30 mins as Trypan Blue is toxic and will lead to an increase in non-viable cells, giving a false cell count.
5. Add 20 µl of the cell suspension mixture to each chamber of a haemocytometer and view under a light microscope.
 - a. Count the number of viable cells (bright cells; non-viable cells take up Trypan Blue readily and thus are dark) in each quadrant of the haemocytometer for a total of 8 quadrants in the upper and lower chamber.

Total cell count is given as (Average number of cells/quadrant) x 10⁴ cells/ml.

d) Staining cells

- i. Preparation before staining cells
 - Cell suspension are aliquot into 3 tubes (CD73, CD90, CD105) in duplicates and 2 tubes (negative control), each containing 50,000 cells.
- ii. Staining with primary antibody (Ab)
 - Add 1 µl [0.5mg/ml Ab] of primary antibody to 100ul cell suspension. Incubate at 4°C for 45 min.
 - Make up to 1ml with PBA.
 - Centrifuge 8000 rpm at 4°C for 5 mins.
 - Remove supernatant.
 - Add 1ml of PBA and re-suspend pellet
 - Centrifuge 8000 rpm at 4°C for 5 mins.
 - Remove supernatant.
 - Re-suspend in 100ul PBA.
- iii. Staining with secondary Ab – in the dark
 - Add 1ul [0.5mg/ml ab] of secondary antibody to 100ul cell suspension. Incubate at 4°C for 30 min.
 - Make up to 1ml with PBA.
 - Centrifuge 8000 rpm at 4°C for 5 mins.

- Remove supernatant.
- Add 1ml of PBA and re-suspend pellet
- Centrifuge 8000 rpm at 4°C for 5 mins.
- Remove supernatant
- Re-suspend in 200-300ul PBA for flow cytometry analysis
- Transfer cells to FACS tube for reading in BD FACS CANDO flow cytometry.

[00173] The results of the flow cytometry analysis are shown in **Fig. 6a** to **Fig.6c**. **Fig. 6a** shows the percentage of isolated mesenchymal cord lining stem cells expressing stem cell markers CD73, CD90 and CD105 after isolation from umbilical cord tissue and cultivation in DMEM/10% FBS, **Fig. 6b** shows the percentage of isolated mesenchymal cord lining stem cells expressing stem cell markers CD73, CD90 and CD105 after isolation from umbilical cord tissue and cultivation in PTT-4 and **Fig. 6c** shows the percentage of isolated mesenchymal cord lining stem cells expressing stem cell markers CD73, CD90 and CD105 after isolation from umbilical cord tissue and cultivation in PTT-6. As can be seen from **Fig. 6a**, the population isolated using DMEM/10 % FBS as culture medium cultivation has about 75% CD73+ cells, 78 % CD90+ cells and 80 % CD105+ cells (average of two experiments), while after isolation/cultivation of umbilical cord tissue using PTT-4 culture medium (see **Fig. 6b**) the number of mesenchymal stem cells that are CD73-positive, CD90-positive and CD105-positive are about 87 % (CD73+ cells), 93 % /CD90+ cells) and 86 % (CD105+ cells) average of two experiments. The purity of the mesenchymal stem cell population that was obtained by means of cultivation in the PTT-6 medium of the present invention is at least 99.0 % with respect to all three markers (CD73, CD90, CD105), meaning the purity of this cell population is significant higher than for cultivation using PTT-4 medium or DMEM/10 % FBS. In addition and even more importantly, the mesenchymal stem cell population obtained by means of cultivation in PTT-6 is essentially a 100% pure and defined stem cell population. This makes the stem cell population of the present invention the ideal candidate for stem cell based therapies. Thus, this population of mesenchymal cord lining stem cells may become the gold standard for such stem cell based therapeutic approaches.

[00174] The findings shown in Fig. 6 are further corroborated by the results of the flow cytometry analysis that are shown in **Fig. 7a** and **Fig.7b**. **Fig. 7a** shows the percentage of isolated mesenchymal cord lining stem cells (mesenchymal stem cells of the amniotic membrane of umbilical cord) that express the stem cell markers CD73, CD90 and CD105 and

lack expression of CD34, CD45 and HLA-DR after isolation from umbilical cord tissue and cultivation in PTT-6 medium. As shown in **Fig. 7a**, the mesenchymal stem cell population contained 97.5 % viable cells of which 100 % expressed each of CD73, CD90 and CD105 (see the rows “CD73+CD90+” and “CD73+CD105+”) while 99.2 % of the stem cell population did not express CD45 and 100 % of the stem cell population did not express CD34 and HLA-DR (see the rows “CD34-CD45-” and “CD34-HLA-DR-”). Thus, the mesenchymal stem cells population obtained by cultivation in PTT-6 medium is essentially a 100% pure and defined stem cell population that meets the criteria that mesenchymal stem cells are to fulfill to be used for cell therapy (95% or more of the stem cell population express CD73, CD90 and CD105, while 98 % or more of the stem cell population lack expression of CD34, CD45 and HLA-DR, see Sensebe et al. “Production of mesenchymal stromal/stem cells according to good manufacturing practices: a review”, *supra*). It is noted here that the present mesenchymal stem cells of the amniotic membrane adhere to plastic in standard culture conditions and differentiate *in vitro* into osteoblasts, adipocytes and chondroblasts, see US patents 9,085,755, US patent 8,287,854 or WO2007/046775 and thus meet the criteria generally accepted for use of mesenchymal stem cells in cellular therapy.

[00175] **Fig. 7b** shows the percentage of isolated bone marrow mesenchymal stem cells that express CD73, CD90 and CD105 and lack expression of CD34, CD45 and HLA-DR. As shown in **Fig. 7b**, the bone marrow mesenchymal stem cell population contained 94.3 % viable cells of which 100 % expressed each of CD73, CD90 and CD105 (see the rows “CD73+CD90+” and “CD73+CD105+”) while only 62.8 % of the bone marrow stem cell population lacked expression of CD45 and 99.9 % of the stem cell population lacked expression CD34 and HLA-DR (see the rows “CD34-CD45-” and “CD34-HLA-DR-”). Thus, the bone marrow mesenchymal stem cells that are considered to be gold standard of mesenchymal stem cells are by far less homogenous/pure in terms of stem cell marker than the mesenchymal stem cells population (of the amniotic membrane of the umbilical cord) of the present application. This finding also shows that the stem cell population of the present invention may be the ideal candidate for stem cell based therapies and may become the gold standard for stem cell based therapeutic approaches.

[00176] **Experiments showing that the mesenchymal stem cell population of the invention can be transported/stored in HypoThermosol™:**

[00177] To analyze health and viability of the mesenchymal stem cells as described herein in different storage or transport carrier, two different carriers were compared to each

other. Namely, the carrier HypoThermosol™-FRS was compared to the carrier PlasmaLyte-A. Both are commercially available. HypoThermosol™-FRS the product sheet of which is shown in Fig. 30 and its composition is described elsewhere herein. Each 100 mL PlasmaLyte 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, USP (MgCl₂•6H₂O). PlasmaLyte does not contain antimicrobial agents. The pH of PlasmaLyte is adjusted with sodium hydroxide to 7.4 (6.5 to 8.0).

[00178] The experimental setup for comparison is shown in **Fig. 8**. First the mesenchymal stem cell population as described herein were outgrown in cell culture flasks. The number of living mesenchymal stem cells was counted and then 2 million cells/vial were stored for different periods of time in either PlasmaLyte-A or Hypothermosol™-FRS. After storage cells have been counted in sample of ≤50µl daily for days 1-5 (total liquid withdrawal 250µl) and checked for viability by staining the cells with Trypan blue. Further, on days 1, 3 and 5 sample ≤80µl were taken and analyzed. In addition, the supernatant was obtained and frozen. Then PDGF-AA, PDGF-BB, VEGF, IL-10, Ang-1, HGF and TGFβ1 were measured by FLEXMAP 3D system.

[00179] **Fig. 9** summarizes viability data. As can be seen from the left-hand graph, 73 % of the total cells (about 95 %) with which the storing started were still viable 7 days after storage in HypoThermosol™. On the contrary after 7 days of storage in PlasmaLyte-A only 42 % of the total of cells (about 94 %) with which the storage started were still viable. All counts based on duplicate readings that are within 10% of one another (following SOP CR D2.600.1). During counting, cells stored in HypoThermosol™ were noticeably smaller with smooth and defined edges. By contrast, cells in Plasmalyte-A appeared of a range of sizes. HypoThermosol™ noticeably supports membrane integrity and presumably survival over a week timespan (6 days). Similar results are also shown in the graph of the right-hand side.

[00180] **Fig. 10** shows the results obtained when measuring the cell diameter of cells. The mesenchymal stem cell population as described herein when kept in HypoThermosol™ are narrower in diameter range when compared to cells kept in PlasmaLyteA. Comparison took place after 3 days of storage.

[00181] **Fig. 11** shows the TGFβ1 concentration in supernatant from the mesenchymal stem cell population as described herein stored in HypoThermosol™ or PlasmaLyte-A after 48 hrs of storage. As can be seen from the graph on the right-hand side, cells secrete about as

much TGF β 1 when stored in HypoThermosolTM and when stored in PlasmaLyte-A. In general, over time, the amount of secreted TGF β 1 decreased (graph on the right hand side).

[00182] **Figs. 12 and 13** show control experiments. Here, the PDGF-BB and IL-10 concentration was measured in supernatant from mesenchymal stem cell population as described herein stored in HypoThermosolTM or PlasmaLyte-A for 48hrs. Since PDGF-BB or IL-10 are not normally secreted by the mesenchymal stem cell population as described herein, no PDGF-BB or IL-10 were detectable in any sample.

[00183] **Fig. 14** shows the VEGF concentration in supernatant from mesenchymal stem cell population as described herein stored in HypoThermosolTM or PlasmaLyte-A for 48 hrs. As can be seen from the graph on the right-hand side, cells secrete about as much VEGF when stored in HypoThermosolTM or PlasmaLyte-A on day 0. On day 1 and 5 cells secreted more VEGF when stored in PlasmaLyte-A. Notably, when stored for 3 days cells secreted more VEGF when stored in HypoThermosolTM than when stored in PlasmaLyte-A. Thus, HypoThermosolTM outperforms PlasmaLyte-A after day 3 of storage. The more VEGF is detected the healthier is the culture. Thus, by secreting more VEGF after 3 days storage in HypoThermosolTM than when stored in PlasmaLyte-A, cells are healthier in HypoThermosolTM than in PlasmaLyte-A. From 5 days of storage onwards PlasmaLyte seems to become a more favourable carrier, because at the time point 5 days, cells stored in PlasmaLyte-A secreted more VEGF. In general, over time, the amount of secreted VEGF decreased (graph on the right hand side).

[00184] **Fig. 15** shows the PDGF-AA concentration in supernatant from mesenchymal stem cell population as described herein stored in HypoThermosolTM or PlasmaLyte-A for 48 hrs. As can be seen from the graph on the right-hand side, cells secrete about as much PDGF-AA when stored in HypoThermosolTM than when stored in PlasmaLyte-A on day 0. On day 1 and 5 cells secreted more PDGF-AA when stored in PlasmaLyte-A. Notably, when stored for 3 days cells, secreted more PDGF-AA when stored in HypoThermosolTM than when stored in PlasmaLyte-A. Thus, cells stored in HypoThermosolTM are healthier than cells stored in PlasmaLyte-A after 3 days of storage. From 5 days of storage onwards, PlasmaLyte seems to become a more favourable carrier, because at the time point 5 days cells stored in PlasmaLyte-A secreted more PDGF-AA. In general over time the amount of secreted PDGF-AA decreased (graph on the right hand side).

[00185] **Fig. 16** shows the Ang-1 concentration in supernatant from mesenchymal stem cell population as described herein stored in HypoThermosolTM or PlasmaLyte-A for 48 hrs.

As can be seen from the graph on the right-hand side, cells secrete about as much Ang-1 when stored in HypoThermosol™ or PlasmaLyte-A on day 0 and 3. On day 5 cells secreted more Ang-1 when stored in PlasmaLyte-A. Notably, when stored for 1 day, cells secreted much more Ang-1 when stored in HypoThermosol™ than when stored in PlasmaLyte-A. Thus, cells stored in HypoThermosol™ seem to be healthier than when stored in PlasmaLyte-A for at least 48 hrs until 3 days of storage. From 5 days of storage onwards PlasmaLyte seems to become a more favourable carrier, because at this time point cells stored in PlasmaLyte-A secreted more Ang-1. In general, over time, the amount of secreted Ang-1 decreased (graph on the right hand side).

[00186] Fig. 17 shows the HGF concentration in supernatant from mesenchymal stem cell population as described herein stored in HypoThermosol™ or PlasmaLyte-A after 48 hrs of storage. As can be seen from the graph on the right-hand side, cells secrete about as much HGF when stored in HypoThermosol™ than when stored in PlasmaLyte-A on day 0. On day 3 and 5 cells secreted more HGF when stored in PlasmaLyte-A. Notably, when stored for 1 day, cells secreted much more HGF when stored in HypoThermosol™ than when stored in PlasmaLyte-A. Thus, cells stored in HypoThermosol™ seem to be healthier than cells stored in PlasmaLyte-A between at least 1 day (48 hrs) until 3 days of storage. From 3 days on PlasmaLyte-A seems to become a more favourable carrier, because at the time points 3 and 5 days cells stored in PlasmaLyte-A secreted more HGF. In general, over time, the amount of secreted HGF decreased (graph on the right hand side).

[00187] In summary from the above data it can be concluded that storage of the mesenchymal stem cell population of the present invention in HypoThermosol™ outperforms storage in PlasmaLyte-A especially for the first 3 days of storage.

[00188] **Experiments showing that the mesenchymal stem cell population of the invention have wound healing properties by topical treatment of pigs:**

[00189] Preclinical studies have also been performed using 10-week old female Yorkshire-Landrace pigs (50 kg). The treatments were performed at SingHealth Experimental Medicine Centre in Singapore. The pigs were rendered diabetic with 120 mg/kg streptozotocin and allowed to recover for 45 days prior to creating six 5 cm x 5 cm full thickness wounds on their backs (*see Fig. 18*). Pigs ($n = 2$) were treated twice weekly with 10^5 human mesenchymal stem cell population as described herein per cm^2 for 4 weeks. The two control pigs were treated with PBS. Wounds were photographed on postoperative day 0 (PODay 0) and every seven days until postoperative Day 35. The wounds were

analyzed for surface area size by ImageJ. By Day 35, the addition of mesenchymal stem cell population as described herein had resulted in closure of 10 of 12 diabetic wounds (83%), compared to only 3 of 12 (25%) of the PBS-treated control wounds. The *rate* of wound healing was 0.8 cm²/day with the mesenchymal stem cell population as described herein compared to 0.6 cm²/day in the control animals, an improvement of 33%. Results of this study are summarized in **Figure 18**.

[00190] The pig model is not spontaneous, but the skin architecture most closely resembles humans. The data suggest that umbilical cord lining mesenchymal stem cell population of the present invention will improve wound healing without the risk of serious adverse side effects. These data thus strongly support the hypothesis that human umbilical cord lining mesenchymal stem cell population as described herein can promote chronic wound healing by suppressing inflammation and promoting angiogenesis. Furthermore, there is clearly no sign of inflammation with the use of xenogeneic mesenchymal stem cells in either mice or pigs, and therefore the likelihood that allogeneic mesenchymal stem cells will have any serious adverse effect in humans is very low.

[00191] **Experiments showing that the mesenchymal stem cells as described herein are effective in topical treatments in humans:**

[00192] Experiments showing that the mesenchymal stem cells as described herein are effective in topical treatments in humans have been described in WO 2007/046775. In particular, as explained in Examples 23-26 of WO 2007/046775 mesenchymal stem cells of the amniotic membrane of the umbilical cord (UCMC) could alleviate full thickness burns (Example 23), partial-thickness wounds (Example 24), non-healing radiation wound (Example 25) as well as non-healing diabetic wound and non-healing diabetic foot wounds (Example 26). Notably, in accordance with Example 2 of WO 2007/046775 mesenchymal stem cells were resuspended in PTT-4 medium.

[00193] Notably, as depicted in Figures 6b and 6c the stem cell population obtained by cultivation when using PTT6 (as used herein) cultivation medium is significantly more homogenous than the population of cells obtained by using PTT4 medium (used in WO 2007/046775). Since PTT-4 was used as medium for mesenchymal stem cells in Examples 23-26 of WO 2007/046775 it is clear that the even more homogenous mesenchymal stem cell population isolated after cultivation in PTT-6 (as used herein) will have the same beneficial effects in wound healing applications, such as full thickness burns, partial-thickness wounds,

non-healing radiation wound as well as non-healing diabetic wound and non-healing diabetic foot wounds.

[00194] Experiments showing that the mesenchymal stem cells as described herein are effective in topical treatments in humans:

[00195] This is a planned study of escalating doses of the mesenchymal stem cell population obtained as described herein performed at the *University of Colorado Anschutz Medical Campus* in Aurora, Colorado. The goal of this study is to determine a safe dose of mesenchymal stem cell population as described herein (human umbilical cord lining mesenchymal stem cells). This is a single-center, dose-escalation study where each of three dose levels will enroll five subjects for a total of fifteen subjects. The first group of 5 patients will receive 100,000 MSC/cm² (skin/wound area) twice per week for 8 weeks. The second group of 5 patients will receive 300,000 MSC/cm² twice per week for 8 weeks. The third group of 5 patients will receive 500,000 MSC/cm² twice per week for 8 weeks. This schedule will continue until either the highest dose is reached, or until at least 2 subjects at a dose level have \geq Grade 2 allergic reaction that is suspected to be related to mesenchymal stem cell population as obtained herein or 2 or more subjects at a dose level experience an unexpected, treatment-related serious adverse event or dose limiting toxicity within 14 days following the initial dose of mesenchymal stem cell population as obtained as described herein. All of the patients will be evaluated 30 days posttreatment for the production of anti-HLA antibodies and for wound closure. At the present time, we do not consider production of HLA antibodies to be an absolute contraindication to a particular dose, but it will factor into our overall assessment of safety. This is an open-label study where all subjects will be taking the study drug and all study personnel will know the dose each subject receives. A secondary endpoint of this study will be significant improvement in the condition of the wound. This endpoint will be based on the rate of wound closure, the percent of wound area successfully closed, and the percent of wounds fully closed, as measured using the Silhouette Wound Measurement and Documentation System. This device is approved by the FDA for this purpose.

[00196] Subject Population. Patients with Type I or Type II diabetes with chronic foot ulcers that have not healed after at least 30 days of conventional therapy and are negative for HLA antibodies to the mesenchymal stem cell population as described herein. Patients will continue with conventional wound treatment for the first 2 weeks commencing at the time of enrollment, at which time they will have already been screened for having a diabetic foot

ulcer that has not healed in 30 days. Photodocumentation and measurement of wound parameters will start at this time. Conventional dressing changes will be performed twice a week for the first 2 weeks, after which mesenchymal stem cell population as described herein will be applied to the wound at the specified concentrations twice a week. The mesenchymal stem cell population as described herein -treated wounds will also be covered with Tegaderm® and a crepe dressing.

[00197] Dose Levels. The goal of this study is to determine a safe dose of human umbilical cord lining mesenchymal stem cells as described herein for further study. Patients will be treated with one of three doses: 100,000 cells/cm² skin/wound area, 300,000 cells/cm² or 500,000 cells/cm² twice a week for 8 weeks. Each 100,000 cell dose represents 0.1 ml of the mesenchymal stem cell population as described herein from a vial containing 1 million cells/ml in HypoThermosol.

[00198] Dosing Regimen. This is a safety and tolerability study of escalating doses of mesenchymal stem cells as described herein. The goal of this study is to determine a safe dose of the human umbilical cord lining mesenchymal stem cells as described herein for further study. Each of three dose levels will enroll five subjects. The first group of 5 patients will receive 100,000 MSC/cm² skin/wound area twice per week for 8 weeks. The second group of 5 patients will receive 300,000 MSC/cm² twice per week for 8 weeks. The third group of 5 patients will receive 500,000 MSC/cm² twice per week for 8 weeks. This schedule will continue until either the highest dose is reached, or until at least 2 subjects at a dose level have ≥ Grade 2 allergic reaction that is suspected to be related to mesenchymal stem cells as described herein or 2 or more subjects at a dose level experience an unexpected, treatment-related serious adverse event or dose limiting toxicity within 30 days following the initial dose of a mesenchymal stem cell population as described herein. All of the patients will be evaluated 30 days posttreatment for the production of anti-HLA antibodies and for degree of wound closure. At the present time, we do not consider production of HLA antibodies to be an absolute contraindication to a particular dose, but it will factor into our overall assessment of safety. This is an open-label study where all subjects will be taking the study drug and all study personnel will know the dose each subject receives.

[00199] Route of Administration. The mesenchymal stem cell population as described herein as described herein are applied topically to debrided diabetic foot ulcers and held in place by a Tegaderm® bandage.

[00200] Dosing Procedure. Following suitable debridement, if needed, the patient is placed in the prone position and the affected leg bent at a 90° angle. This vial of the mesenchymal stem cell population as described herein is gently swirled to ensure equal distribution of the cells. The elevated foot is then treated by removing 100,000 (0.1 ml) to 500,000 (0.5 ml) cells per cm² from the vial using a sterile syringe and placing it in the center of the wound. The wound is then sealed with a Tegaderm® membrane and gently massaged to distribute the cells evenly. The foot is maintained elevated for five minutes to allow the cells to settle and attach. The foot is then dressed with a crepe bandage to cover the Tegaderm® dressing.

[00201] The invention is further characterized by the following items:

1. A method of transporting a stem cell population, the method comprising transporting said stem cell population contacted with a liquid carrier, said liquid carrier comprising
 - i) Trolox;
 - ii) Na⁺;
 - iii) K⁺;
 - iv) Cl⁻;
 - v) H₂PO₄⁻;
 - vi) HEPES;
 - vii) Lactobionate;
 - viii) Sucrose;
 - ix) Mannitol;
 - x) Glucose;
 - xi) Dextran-40;
 - xii) Adenosine, and
 - xiii) Glutathione.
2. The method of item 1, wherein the transporting is performed for about 7 days or less.
3. The method of item 1 or 2, wherein the transporting is performed for about 6 days, about 5 days, about 4 days, about 3 days, about 2 days, about 1 day or for less than about 1 day.
4. The method of any one of the preceding items, wherein the transporting is performed for about 48 hours or about 24 hours or less.

5. The method of any one of the foregoing items, wherein the transporting is performed at a temperature of about -5°C to about 15°C .
6. The method of any one of the foregoing items, wherein the transporting is performed at a temperature of about 2°C to about 8°C .
7. The method of any one of the foregoing items, wherein the transporting is carried out at a temperature of more than about -5°C , more than about -10°C , more than about -15°C , or more than about -20°C .
8. The method of any one of the foregoing items, wherein the stem cell population is transported in a concentration of about 70 million cells per 1 ml carrier, of about 60 million cells per 1 ml carrier, of about 50 million cells per 1 ml carrier, of about 40 million cells per 1 ml carrier, of about 30 million cells per 1 ml carrier, of about 20 million cells per 1 ml carrier, of about 10 million cells per 1 ml carrier, of about 5 million cells per 1 ml carrier, of about 4 million cells per 1 ml carrier, of about 3 million cells per 1 ml carrier, of about 2 million cells per 1 ml carrier, of about 1 million cells per 1 ml carrier, of about 0.5 million cells per 1 ml carrier, of about 0.1 million cells per 1 ml carrier or of less than 0.1 million cells per 1 ml carrier.
9. The method of item 8, wherein the stem cell population is transported in a concentration of about 10 million cells per ml carrier to about 1 million cells per 1 ml carrier.
10. The method of any one of the foregoing items, wherein the stem cell population is an embryonic stem cell population, an adult stem cell population, a mesenchymal stem cell population or an induced pluripotent stem cell population.
11. The method of any one of the foregoing items, wherein the stem cell population is a mesenchymal stem cell population.
12. The method of any one of the foregoing items, wherein the mesenchymal stem cell population is an isolated mesenchymal stem population of the amniotic membrane of the umbilical cord.

13. The method of item 11 or 12, wherein at least about 90 % or more cells of the isolated mesenchymal stem cell population express each of the following markers: CD73, CD90 and CD105.
14. The method of item 13, wherein at least about 91 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more cells of the isolated mesenchymal stem cell population express each of CD73, CD90 and CD105.
15. The method of any one of items 11-14, wherein at least about 90 % or more, about 91 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more of the isolated mesenchymal stem cells lack expression of the following markers: CD34, CD45 and HLA-DR (Human Leukocyte Antigen – antigen D Related).
16. The method of any one of the foregoing items, wherein the stem cell population is contacted with the carrier before transporting.
17. The method of any one of the foregoing items, wherein the stem cell population is contacted with the carrier after its harvest.
18. The method of item 17, wherein the stem cell population is contacted with the carrier about 0 minutes, about 1 minute, about 5 minutes, about 10 minutes, about 30 minutes, about 45 minutes, about 60 minutes or a longer time after its harvest.
19. The method of item 17 or 18, wherein the harvest comprises separating the stem cell population from culture medium.
20. The method of item 19, wherein separating is performed by centrifuging the stem cells within a culture medium and decanting the culture medium.
21. The method of any one of the foregoing items, wherein the contacting is performed by suspending the stem cell population in a density of about 70 million/ml, of about 60 million/ml, of about 50 million/ml, of about 40 million/ml, of about 30 million/ml, of about 20 million/ml, of about 10 million/ml, of about 5 million/ml, of about 4 million/ml, of about 3 million/ml, of about 2 million/ml, of about 1 million/ml, of

- about 0.5 million/ml, of about 0.1 million/ml or of less than 0.1 million cells in the carrier.
22. The method of item 21, wherein the contacting is performed by suspending the stem cell population in a density of about 10 million/ml in the carrier.
 23. The method of item 21 or 22, wherein the stem cells contacted with the carrier are aliquoted into vials in a volume of about 50 ml, of about 20 ml, of about 10 ml, of about 5 ml, of about 4 ml, of about 3 ml, of about 2 ml, of about 1 ml, of about 0.5 ml, of about 0.25 ml or of less than 0.25 ml carrier.
 24. The method of item 21 or 22, wherein the stem cells contacted with the carrier are aliquoted into vials in an amount of about 1 ml.
 25. The method of any one of the foregoing items, wherein the carrier is a transport medium or an excipient.
 26. The method of any one of the foregoing items, wherein the method does not comprise a thawing or freezing step.
 27. The method of any one of the foregoing items, wherein the carrier does not include a dipolar aprotic solvent, in particular DMSO.
 28. The method of any one of the foregoing items, wherein at most about 50 %, about 40 %, about 30 %, about 20 %, about 10 % or less than about 10 % of the stem cells of the population die during transporting compared to the amount of viable stem cells before transporting.
 29. The method of any one of the foregoing items, wherein most of the stem cells in the stem cell population have a cell diameter between about 9 μm and about 20 μm after transporting.
 30. The method of any one of the foregoing items, wherein most of the stem cells in the stem cell population have a cell diameter between about 12 μm and about 16 μm after transporting.
 31. The method of any one of the foregoing items, wherein after transport the stem cell population secretes about as much TGFbeta-1 as before transporting.

32. The method of any one of the foregoing items, wherein essentially no PDGF-BB and/or IL-10 is detected before and/or after transporting.
33. The method of any one of the foregoing items, wherein after transporting the mesenchymal stem cell population secretes about as much VEGF, PDGF-AA, Ang-1, and/or HGF as before transporting.
34. A method of treating a subject having a disease, the method comprising topically administering a mesenchymal stem cell population as defined in any one of items 12-15 to the subject, wherein the mesenchymal stem cell population is administered within about 96 hours from the time point the mesenchymal stem cell population has been harvested.
35. The method of item 34, wherein the mesenchymal stem cell population is applied in a unit dosage of about 20 million cells, of about 15 million cells, of about 10 million cells, of about 5 million cells, of about 4 million cells, of about 3 million cells, of about 2 million cells, of about 1 million cells, of about 0.5 million cells, of about 0.25 million cells or of less than 0.25 million cells.
36. The method of item 34 or 35, wherein the mesenchymal stem cell population is applied in a unit dosage of about 10 million cells.
37. The method of any one of items 34-36, wherein the mesenchymal stem cell population is applied within about 72 hours, about 48 hours, about 24 hours, about 12 hours, about 6 hours or less from the time point the mesenchymal stem cell population has been harvested.
38. The method of any one of items 34-37, wherein the disease is a skin disease or a wound.
39. The method of item 38, wherein the wound is caused by a burn, a bite, a trauma, a surgery, or a disease.
40. The method of item 39, wherein the wound is caused by diabetic disease, wherein the wound is preferably a diabetic wound.
41. The method of item 40, wherein the wound is diabetic foot ulcer.

42. The method of any of items 34 to 41, wherein the unit dosage of about 20 million cells, of about 15 million cells, of about 10 million cells, of about 5 million cells, of about 4 million cells, of about 3 million cells, of about 2 million cells, of about 1 million cells, of about 0.5 million cells, of about 0.25 million cells or of less than 0.25 million cells is administered once or twice a week.
43. The method of item 42, wherein the unit dosage of about 20 million cells, of about 15 million cells, of about 10 million cells, of about 5 million cells, of about 4 million cells, of about 3 million cells, of about 2 million cells, of about 1 million cells, of about 0.5 million cells, of about 0.25 million cells or of less than 0.25 million cells is administered one of twice a week for a period of time of three weeks, of four weeks, or five weeks or of six weeks, or of seven weeks, or of eight weeks or of ten weeks or more weeks.
44. The method of any one of items 34-43, wherein the mesenchymal stem cell population is applied topically and covered by a film or bandage.
45. The method of any one of items 34-44, wherein the mesenchymal stem cell population is applied in a dosage of about 1000 cells/cm² to about 5 million cells/cm².
46. The method of any one of items 34-45, wherein the mesenchymal stem cell population is applied in a dosage of about 100,000 cells/cm², of about 300,000 cells/cm² or of about 500,000 cells/cm².
47. The method of any one of items 34-46, wherein the mesenchymal stem cell population is applied once, twice or more times a week.
48. The method of any one of items 34-47, wherein the mesenchymal stem cell population is applied for one, two, three, four, five, six, seven, eight, nine, ten, eleven weeks or more.
49. The method of any one of items 34-48, wherein the mesenchymal stem cell population is applied two times a week for about 8 weeks in a dosage of about 100,000 cells/cm², about 300,000 cells/cm² or about 500,000 cells/cm².

50. The method of any of items 34 to 49, wherein the mesenchymal stem cell population is administered to the subject after separating the mesenchymal stem cell population from the carrier.
51. The method of item 50, wherein separating the mesenchymal stem cell population from the carrier comprises centrifugation.
52. The method of item 50 and 51, separating the mesenchymal stem cell population from the carrier comprises withdrawing the cell population from the vial by means of syringe.
53. The method of any of items 34 to 52, comprising administering the mesenchymal stem cell population by means of a syringe.
54. A unit dosage comprising about 20 million cells, of about 15 million cells, of about 10 million cells, of about 5 million cells, of about 4 million cells, of about 3 million cells, of about 2 million cells, of about 1 million cells, of about 0.5 million cells, of about 0.25 million cells or of less than 0.25 million cells of a mesenchymal stem cell population as defined in any one of items 12-15.
55. The unit dosage of item 54, wherein the unit dosage comprises about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2, about 1, about 0.5, about 0.25, or about 0.1 million cells.
56. The unit dosage of item 54 or 55, wherein the unit dosage comprises about 10 million cells.
57. The unit dosage of any one of items 54-56, wherein the unit dosage comprises about 1000 cells to about 5 million cells.
58. The unit dosage of any one of items 54-57, wherein the unit dosage is applied in a dosage of about 100,000 cells, 300,000 cells or 500,000 cells.
59. The unit dosage of any one of items 54-58, wherein the unit dosage is applied topically.
60. The unit dosage of any one of items 54-59, wherein the unit dosage is applied topically per cm².

61. The unit dosage of any one of items 54-60, wherein the unit dosage is applied once, twice, three times or more times a week.
62. The unit dosage of any one of items 54-61, wherein the unit dosage is applied for one, two, three, four, five, six, seven, eight, nine, ten, eleven weeks or more.
63. The unit dosage of any one of items 54-62, wherein the unit dosage comprises of about 100,000 cells, about 300,000 cells or about 500,000 cells of any one of items is applied two times a week for 8 weeks.
64. The unit dosage of any one of items 54-63, wherein the unit dosage is contained in a 1 ml vial.
65. The unit dosage of item 64, wherein 0.1 ml of the vial are applied.
66. The unit dosage of any one of items 54 to 65, wherein the cells are in contact with a liquid carrier as defined in item 1.
67. The unit dosage of any one of items 54 to 66, wherein the cells are centrifuged and isolated before administration to a subject.
68. The unit dosage of any one of items 54 to 67, wherein the carrier is HypoThermosol™.
69. The method of any of items 34 to 53 and the unit dosage of any one of items 54-68 wherein the cells are viable cells.
70. The method of any of items 1 to 33, wherein the carrier is HypoThermosol.
71. The use of a liquid carrier for transporting a stem cell population, wherein the liquid carrier comprises
 - i) Trolox;
 - ii) Na+;
 - iii) K+;
 - iv) Cl-;
 - v) H₂PO₄-;
 - vi) HEPES;

- vii) Lactobionate;
 - viii) Sucrose;
 - ix) Mannitol;
 - x) Glucose;
 - xi) Dextran-40;
 - xii) Adenosine, and
 - xiii) Glutathione.
72. The use of item 71, wherein the transporting is performed for about 7 days or less.
73. The use of item 71 or 72, wherein the transporting is performed for about 6 days, about 5 days, about 4 days, about 3 days, about 2 days, about 1 day or for less than about 1 day.
74. The use of any one of the preceding items 71 to 73, wherein the transporting is performed for about 48 hours or about 24 hours or less.
75. The use of any one of the foregoing items 71 to 74, wherein the transporting is performed at a temperature of about -5°C to about 15°C .
76. The use of any one of the foregoing items 71 to 75, wherein the transporting is performed at a temperature of about 2°C to about 8°C .
77. The use of any one of the foregoing items 71 to 76, wherein the transporting is carried out at a temperature of more than about -5°C , more than about -10°C , more than about -15°C , or more than about -20°C .
78. The use of any one of the foregoing items 71 to 77, wherein the stem cell population is transported in a concentration of about 70 million cells per 1 ml carrier, of about 60 million cells million cells per 1 ml carrier, of about 50 million cells per 1 ml carrier, of about 40 million cells per 1 ml carrier, of about 30 million cells per 1 ml carrier, of about 20 million cells per 1 ml carrier, of about 10 million cells per 1 ml carrier, of about 5 million cells per 1 ml carrier, of about 4 million cells per 1 ml carrier, of about 3 million cells per 1 ml carrier, of about 2 million cells per 1 ml carrier, of about 1 million cells per 1 ml carrier, of about 0.5 million cells per 1 ml carrier, of about 0.1 million cells per 1 ml carrier or of less than 0.1 million cells per 1 ml carrier.

79. The use of item 78, wherein the stem cell population is transported in a concentration of about 10 million cells per 1 ml carrier to about 1 million cells per 1 ml carrier.
80. The use of any one of the foregoing items 71 to 79, wherein the stem cell population is an embryonic stem cell population, an adult stem cell population, a mesenchymal stem cell population or an induced pluripotent stem cell population.
81. The use of any one of the foregoing items 71 to 80, wherein the stem cell population is a mesenchymal stem cell population.
82. The use of any one of the foregoing items 71 to 81, wherein the mesenchymal stem cell population is an isolated mesenchymal stem population of the amniotic membrane of the umbilical cord.
83. The use of item 81 or 82, wherein at least about 90 % or more cells of the isolated mesenchymal stem cell population express each of the following markers: CD73, CD90 and CD105.
84. The use of item 83, wherein at least about 91 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more cells of the isolated mesenchymal stem cell population express each of CD73, CD90 and CD105.
85. The use of any one of items 81-84, wherein at least about 90 % or more, about 91 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more of the isolated mesenchymal stem cells lack expression of the following markers: CD34, CD45 and HLA-DR (Human Leukocyte Antigen – antigen D Related).
86. The use of any one of the foregoing items 71 to 85, wherein the stem cell population is contacted with the carrier before transporting.
87. The use of any one of the foregoing items 71 to 86, wherein the stem cell population is contacted with the carrier after its harvest.

88. The use of item 87, wherein the stem cell population is contacted with the carrier about 0 minutes, about 1 minute, about 5 minutes, about 10 minutes, about 30 minutes, about 45 minutes, about 60 minutes or a longer time after its harvest.
89. The use of item 87 or 88, wherein the harvest comprises separating the stem cell population from culture medium.

[00202] It will be readily apparent to a person skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

[00203] All patents and publications mentioned in the specification are indicative of the levels of those of ordinary skill in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

[00204] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention. The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group. Further embodiments of the invention will become apparent from the following claims.

[00205] When used herein, the term "about" is understood to mean that there can be variation in the respective value or range (such as pH, concentration, percentage, molarity, number of amino acids, time etc.) that can be up to 5%, up to 10%, up to 15% or up to and including 20% of the given value. For example, if a formulation comprises about 5 mg/ml of a compound, this is understood to mean that a formulation can have between 4 and 6 mg/ml, preferably between 4.25 and 5.75 mg/ml, more preferably between 4.5 and 5.5 mg/ml and even more preferably between 4.75 and 5.25 mg/ml, with the most preferred being 5 mg/ml. As used herein, an interval which is defined as "(from) X to Y" equates with an interval which is defined as "between X and Y". Both intervals specifically include the upper limit and also the lower limit. This means that for example an interval of "5 mg/ml to 10 mg/ml" or "between 5 mg/ml and 10 mg/ml" includes a concentration of 5, 6, 7, 8, 9, and 10 mg/ml as well as any given intermediate value.

What is claimed is

1. A method of transporting a stem cell population, the method comprising transporting said stem cell population contacted with a liquid carrier, said liquid carrier comprising
 - i) Trolox;
 - ii) Na⁺;
 - iii) K⁺;
 - iv) Cl⁻;
 - v) H₂PO₄⁻;
 - vi) HEPES;
 - vii) Lactobionate;
 - viii) Sucrose;
 - ix) Mannitol;
 - x) Glucose;
 - xi) Dextran-40;
 - xii) Adenosine, and
 - xiii) Glutathione.
2. The method of claim 1, wherein the transporting is performed for about 7 days or less.
3. The method of claim 1 or 2, wherein the transporting is performed for about 6 days, about 5 days, about 4 day, about 3 day, about 2 day, about 1 day or for less than about 1 day.
4. The method of any one of the preceding claims, wherein the transporting is performed for about 48 hours or about 24 hours or less.
5. The method of any one of the foregoing claims, wherein the transporting is performed at a temperature of about -5°C to about 15°C.
6. The method of any one of the foregoing claims, wherein the transporting is performed at a temperature of about 2°C to about 8°C.
7. The method of any one of the foregoing claims, wherein the transporting is carried out at a temperature of more than about -5°C, more than about -10°C , more than about -15°C , or more than about -20°C.

8. The method of any one of the foregoing claims, wherein the stem cell population is transported in a concentration of about 70 million cells per 1 ml carrier, of about 60 million cells million cells per 1 ml carrier, of about 50 million cells per 1 ml carrier, of about 40 million cells per 1 ml carrier, of about 30 million cells per 1 ml carrier, of about 20 million cells per 1 ml carrier, of about 10 million cells per 1 ml carrier, of about 5 million cells per 1 ml carrier, of about 4 million cells per 1 ml carrier, of about 3 million cells per 1 ml carrier, of about 2 million cells per 1 ml carrier, of about 1 million cells per 1 ml carrier, of about 0.5 million cells per 1 ml carrier, of about 0.1 million cells per 1 ml carrier or of less than 0.1 million cells per 1 ml carrier.
9. The method of claim 8, wherein the stem cell population is transported in a concentration of about 10 million cells per ml carrier to about 1 million cells per 1 ml carrier.
10. The method of any one of the foregoing claims, wherein the stem cell population is an embryonic stem cell population, an adult stem cell population, a mesenchymal stem cell population or an induced pluripotent stem cell population.
11. The method of any one of the foregoing claims, wherein the stem cell population is a mesenchymal stem cell population, preferably a human mesenchymal stem cell population.
12. The method of any one of the foregoing claims, wherein the mesenchymal stem cell population is an isolated mesenchymal stem population of the amniotic membrane of the umbilical cord, preferably an isolated human mesenchymal stem population of the amniotic membrane of the umbilical cord.
13. The method of claim 11 or 12, wherein at least about 90 % or more cells of the isolated mesenchymal stem cell population express each of the following markers: CD73, CD90 and CD105.
14. The method of claim 13, wherein at least about 91 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more cells of the isolated mesenchymal stem cell population express each of CD73, CD90 and CD105.

15. The method of any one of claims 11-14, wherein at least about 90 % or more, about 91 % or more, about 92 % or more, about 93 % or more, about 94 % or more, about 95 % or more, about 96 % or more, about 97 % or more, about 98 % or more about 99 % or more of the isolated mesenchymal stem cells lack expression of the following markers: CD34, CD45 and HLA-DR (Human Leukocyte Antigen – antigen D Related).
16. The method of claim 15, wherein at least 97 % or more, about 98 % or more or about 99 % or more of the cells of the isolated mesenchymal stem cell population express each of CD73, CD90 and CD105 and lack expression of each of CD34, CD45 and HLA-DR.
17. The method of any one of the foregoing claims, wherein the stem cell population is contacted with the carrier before transporting.
18. The method of any one of the foregoing claims, wherein the stem cell population is contacted with the carrier after its harvest.
19. The method of claim 18, wherein the stem cell population is contacted with the carrier about 0 minutes, about 1 minute, about 5 minutes, about 10 minutes, about 30 minutes, about 45 minutes, about 60 minutes or a longer time after its harvest.
20. The method of claim 18 or 19, wherein the harvest comprises separating the stem cell population from culture medium.
21. The method of claim 20, wherein separating is performed by centrifuging the stem cells within a culture medium and decanting the culture medium.
22. The method of any one of the foregoing claims, wherein the contacting is performed by suspending the stem cell population in a density of about 70 million/ml, of about 60 million/ml, of about 50 million/ml, of about 40 million/ml, of about 30 million/ml, of about 20 million/ml, of about 10 million/ml, of about 5 million/ml, of about 4 million/ml, of about 3 million/ml, of about 2 million/ml, of about 1 million/ml, of about 0.5 million/ml, of about 0.1 million/ml or of less than 0.1 million cells in the carrier.
23. The method of claim 22, wherein the contacting is performed by suspending the stem cell population in a density of about 10 million/ml in the carrier.

24. The method of claim 22 or 23, wherein the stem cells contacted with the carrier are aliquoted into vials in a volume of about 50 ml, of about 20 ml, of about 10 ml, of about 5 ml, of about 4 ml, of about 3 ml, of about 2 ml, of about 1 ml, of about 0.5 ml, of about 0.25 ml or of less than 0.25 ml carrier.
25. The method of claim 22 or 23, wherein the stem cells contacted with the carrier are aliquoted into vials in an amount of about 1 ml.
26. The method of any one of the foregoing claims, wherein the carrier is a transport medium or an excipient.
27. The method of any one of the foregoing claims, wherein the method does not comprise a thawing or freezing step.
28. The method of any one of the foregoing claims, wherein the carrier does not include a dipolar aprotic solvent, in particular DMSO.
29. The method of any one of the foregoing claims, wherein at most about 50 %, about 40 %, about 30 %, about 20 %, about 10 % or less than about 10 % of the stem cells of the population die during transporting compared to the amount of viable stem cells before transporting.
30. The method of any one of the foregoing claims, wherein after transport the stem cell population secretes about as much TGFbeta-1 as before transporting.
31. The method of any one of the foregoing claims, wherein essentially no PDGF-BB and/or IL-10 is detected before and/or after transporting.
32. The method of any one of the foregoing claims, wherein after transporting the mesenchymal stem cell population secretes about as much VEGF, PDGF-AA, Ang-1, and/or HGF as before transporting.
33. A unit dosage comprising about 20 million cells, of about 15 million cells, of about 10 million cells, of about 5 million cells, of about 4 million cells, of about 3 million cells, of about 2 million cells, of about 1 million cells, of about 0.5 million cells, of about 0.25 million cells or of less than 0.25 million cells of a mesenchymal stem cell population as defined in any one of claims 12-16.

34. The unit dosage of claim 33, wherein the unit dosage comprises about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2, about 1, about 0.5, about 0.25, or about 0.1 million cells.
35. The unit dosage of claim 33 or 34, wherein the unit dosage comprises about 10 million cells.
36. The unit dosage of any one of claims 33 to 35, wherein the unit dosage is contained in a 1 ml vial.
37. The unit dosage of any one of claims 33 to 35, wherein the unit dose is contained in a liquid carrier, wherein the liquid carrier comprises
 - i) Trolox;
 - ii) Na+;
 - iii) K+;
 - iv) Cl-;
 - v) H₂PO₄⁻;
 - vi) HEPES;
 - vii) Lactobionate;
 - viii) Sucrose;
 - ix) Mannitol;
 - x) Glucose;
 - xi) Dextran-40;
 - xii) Adenosine, and
 - xiii) Glutathione.
38. The unit dosage of any one of claims 33 to 37, wherein the cells are viable cells.
39. The method of any of claims 1 to 32, wherein the carrier is HypoThermosol.
40. The use of a liquid carrier for transporting a stem cell population, wherein the liquid carrier comprises
 - i) Trolox;
 - ii) Na+;
 - iii) K+;
 - iv) Cl-;
 - v) H₂PO₄⁻;
 - vi) HEPES;

- vii) Lactobionate;
 - viii) Sucrose;
 - ix) Mannitol;
 - x) Glucose;
 - xi) Dextran-40;
 - xii) Adenosine, and
 - xiii) Glutathione.
41. The use of a mesenchymal stem cell population, wherein the mesenchymal stem cell population is an isolated mesenchymal stem population of the amniotic membrane of the umbilical cord as defined in any one of claims 12-16, for the manufacture of a pharmaceutical composition for treating a subject having a disease, wherein the use comprises that the mesenchymal stem cell population is be topically administered within about 96 hours from the time point the mesenchymal stem cell population has been harvested.
42. The use of claim 41, wherein the mesenchymal stem cell population is applied in a unit dosage of about 10 million cells.
43. The use of claim 41 or 42, wherein the mesenchymal stem cell population is applied within about 72 hours, about 48 hours, about 24 hours, about 12 hours, about 6 hours or less from the time point the mesenchymal stem cell population has been harvested.
44. The use of any one of claims 41 to 43, wherein the disease is a skin disease or a wound.
45. The use of claim 44, wherein the wound is caused by a burn, a bite, a trauma, a surgery, or a disease.
46. The use of claim 45, wherein the wound is caused by diabetic disease, wherein the wound is preferably a diabetic wound.
47. The use of claim 46, wherein the wound is diabetic foot ulcer.
48. The use of any one of claims 41 to 47, wherein the unit dosage of about 20 million cells, of about 15 million cells, of about 10 million cells, of about 5 million cells, of about 4 million cells, of about 3 million cells, of about 2 million cells, of about 1

million cells, of about 0.5 million cells, of about 0.25 million cells or of less than 0.25 million cells is administered once or twice a week.

49. The use of claim 48, wherein the unit dosage of about 20 million cells, of about 15 million cells, of about 10 million cells, of about 5 million cells, of about 4 million cells, of about 3 million cells, of about 2 million cells, of about 1 million cells, of about 0.5 million cells, of about 0.25 million cells or of less than 0.25 million cells is administered one of twice a week for a period of time of three weeks, of four weeks, or five weeks or of six weeks, or of seven weeks, or of eight weeks or of ten weeks or more weeks.
50. The use of any one of claims 41 to 49, wherein the mesenchymal stem cell population is applied topically and covered by a film or bandage.

Fig. 1

Lonza

www.lonza.com
 U.S. Scientific Support: 800-521-0390
 scientific.support@lonza.com
 EU/ROW Scientific Support: +49-221-99199-400
 scientific.support.eu@lonza.com
 Document # TS-12-604-3 08/11
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Dulbecco's modified eagle medium (DMEM)

Product use

Dulbecco's modified eagle medium was developed in 1969 and is a modification of basal medium eagle (BME) that differs from BME and MEM by the following characteristics:

- Vitamins 4X greater than MEM. Vitamins and amino acids greater than BME
- Types and quantities of amino acids greater than MEM and BME
- Iron (ferric nitrate)

Description

| | |
|--------|--|
| 12-604 | With 4.5 g/L glucose, with L-glutamine |
| 12-614 | With 4.5 g/L glucose, without L-glutamine |
| 12-707 | With 1.0 g/L glucose, without L-glutamine |
| 12-708 | With 1.0 g/L glucose, and 25 mM HEPES buffer, without L-glutamine |
| 12-709 | With 4.5 g/L glucose, and 25 mM HEPES buffer, without L-glutamine |
| 12-733 | With 4.5 g/L glucose, without L-glutamine, without sodium pyruvate |
| 12-741 | With 4.5 g/L glucose, with L-glutamine, without sodium pyruvate |
| 12-914 | With 4.5 g/L glucose, without L-glutamine, screened to support hybridoma growth |
| 12-917 | With 4.5 g/L glucose, without L-glutamine or phenol red |
| 15-604 | With 4.5 g/L glucose, with L-glutamine, without sodium pyruvate, powder |
| 15-614 | With 4.5 g/L glucose, without L-glutamine or sodium pyruvate, powder (Powder formulations require the addition of 49.3 ml/L of NaHCO ₃ 7.5% solution or 3.70 g/L of NaHCO ₃ powder) |

Quick reference chart

| | Glucose | L-glutamine | Phenol red | HEPES buffer | Sodium pyruvate |
|--------|---------|-------------|------------|--------------|-----------------|
| 12-604 | 4.5 g/L | + | + | - | + |
| 12-614 | 4.5 g/L | - | + | - | + |
| 12-707 | 1.0 g/L | - | + | - | + |
| 12-708 | 1.0 g/L | - | + | + | + |
| 12-709 | 4.5 g/L | - | + | + | + |
| 12-733 | 4.5 g/L | - | + | - | - |
| 12-741 | 4.5 g/L | + | + | - | - |
| 12-914 | 4.5 g/L | - | + | - | + |
| 12-917 | 4.5 g/L | - | - | - | + |

Fig. 1 continued



Specifications

| | Sterility | pH | Osmolality (mOsm) | Cell growth promotion (% of control) | Endotoxin (EU/ml) |
|------------|-----------|-----------|-------------------|--------------------------------------|-------------------|
| 12-604 | Neg. | 7.0-7.4 | 324-352 | ≥75% | FIO |
| 12-614 | Neg. | 7.0-7.4 | 324-352 | ≥75% | FIO |
| 12-707 | Neg. | 7.0-7.4 | 306-346 | ≥75% | FIO |
| 12-708 | Neg. | 7.03-7.27 | 300-326 | ≥75% | FIO |
| 12-709 | Neg. | 7.0-7.4 | 321-351 | ≥75% | FIO |
| 12-733 | Neg. | 7.0-7.4 | 318-360 | ≥75% | FIO |
| 12-741 | Neg. | 7.0-7.4 | 318-360 | ≥75% | FIO |
| 12-914 | Neg. | 7.0-7.4 | 324-352 | ≥85% ** | FIO |
| 12-917 | Neg. | 7.0-7.4 | 329-343 | ≥75% | FIO |
| 15-604 *** | - | 5.5-7.0 | 235-265 | ≥75% | ≤1.0 |
| 15-614 *** | - | 5.5-7.0 | 235-265 | ≥75% | ≤1.0 |

FIO – for information only

** Result of functional test

*** Moisture content #1.0

Storage

2°C to 8°C

Product use statement

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

Ordering information

| Catalog number | Description | Size |
|----------------|---|---------|
| 12-604F | DMEM with 4.5 g/L glucose, with L-glutamine | 500 ml |
| 12-604Q | DMEM with 4.5 g/L glucose, with L-glutamine | 1 L |
| 12-614F | DMEM with 4.5 g/L glucose, without L-glutamine | 500 ml |
| 12-614Q | DMEM with 4.5 g/L glucose, without L-glutamine | 1 L |
| 12-707F | DMEM with 1.0 g/L glucose, without L-glutamine | 500 ml |
| 12-708F | DMEM with 1.0 g/L glucose, and 25 mM HEPES buffer, without L-glutamine | 500 ml |
| 12-709F | DMEM with 4.5 g/L glucose, and 25 mM HEPES buffer, without L-glutamine | 500 ml |
| 12-733F | DMEM with 4.5 g/L glucose, without L-glutamine, without sodium pyruvate | 500 ml |
| 12-733Q | DMEM with 4.5 g/L glucose, without L-glutamine, without sodium pyruvate | 1 L |
| 12-741F | DMEM with 4.5 g/L glucose, with L-glutamine, without sodium pyruvate | 500 ml |
| 12-914F | DMEM with 4.5 g/L glucose, without L-glutamine, screened to support hybridoma growth. | 500 ml |
| 12-917F | DMEM with 4.5 g/L glucose, without L-glutamine or phenol red. | 500 ml |
| 15-604D | DMEM with 4.5 g/L glucose, with L-glutamine, without sodium pyruvate, powder | 1 x 10L |
| 15-604F | DMEM with 4.5 g/L glucose, without L-glutamine or sodium pyruvate, powder | 1 X 50L |
| 15-614D | DMEM with 4.5 g/L glucose, without L-glutamine or sodium pyruvate, powder | 1 x 10L |

Fig. 2

Lonza

Lonza Walkersville, Inc.
 www.lonza.com
 biotechserv@lonza.com
 Tech Service: 800-521-0390
 Document # TS 12-615-2 11/08
 Walkersville, MD 21793-0127 USA
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Ham's F12 Medium

Product Use

Ham's F12 Medium is a nutrient mixture designed to cultivate a wide variety of mammalian and hybridoma cells when used with serum in combination with hormones and transferrin.

Specifications

| | | |
|-----------------------------------|------------------------------|------------------------------|
| Sterility | pH | Osmolality (mOsm) |
| Negative | 7.07-7.40 | 286-305 |
| Cell Growth Generation | Endotoxin (EU/ml) | |
| ≥75% of control | FIO | |

Storage

2°C to 8°C

Product Use Statement

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

Ordering Information

| Catalog Number | Description | Size |
|----------------|-----------------------------------|--------|
| 12-615F | Ham's F12 Medium with L-glutamine | 500 ml |

Fig. 3



www.lonza.com
 U.S. Scientific Support: 800-521-0390
 scientific.support@lonza.com
 EU/ROW Scientific Support: +49-221-99199-400
 scientific.support.eu@lonza.com
 Document # TS-12-719-3 08/11
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DMEM: F12 (1:1) medium

Product use

DMEM combined with Hams F12 has been extensively used to demonstrate the effect of various hormones and growth factors on target tissues.

Description

- 12-719 with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose
- 15-719 powder – with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose. Powdered medium requires the addition of 16.0 ml/L of NaHCO₃, 7.5% solution or 1.2 g/L NaHCO₃ powder
- 04-687 with 3.151 g/L glucose, with L-glutamine, without HEPES

Specifications

| | Sterility | pH | Osmolality (mOsm) |
|---------|-----------------------|-------------------|-------------------|
| 12-719 | Negative | 7.0-7.4 | 286-356 |
| 15-719* | Negative | 4.5-6.5 | 260-290 |
| 04-687 | Negative | FIO | FIO |
| | Cell growth promotion | Endotoxin (EU/ml) | Moisture |
| 12-719 | ≥ 75% of control | FIO | -- |
| 15-719* | ≥ 75% of control | ≤ 1 EU/ml | ≤ 2% |
| 04-687 | -- | FIO | -- |

* pH, osmolality, endotoxin, and moisture are done without NaHCO₃; NaHCO₃ is added for cell growth test.

Storage

2°C to 8°C

Product use statement

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

Ordering information

| Catalog number | Description | Size |
|----------------|--|---------|
| 12-719F | DMEM: F12 with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose | 500 ml |
| 12-719Q | DMEM: F12 with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose | 1 L |
| 15-719D | DMEM: F12 powder – with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose. Powdered medium requires the addition of 16.0 ml/L of NaHCO ₃ , 7.5% solution or 1.2 g/L NaHCO ₃ powder | 1 x 10L |
| 04-687Q | DMEM: F12 with 3.151 g/L glucose, with L-glutamine, without HEPES | 1 L |

Fig. 4

Cascade Biologics™
invitrogen cell culture



Medium 171, Medium 171PRF, and MEGS

Medium 171

M-171-500

500 ml

Medium 171PRF (Phenol Red-Free)

M-171PRF-500

500 ml

Product Description

Medium 171 and Medium 171PRF are sterile, liquid tissue culture media intended for use as one component in a complete culture environment for the growth of normal human mammary epithelial cells. Medium 171 is a basal medium containing essential and non-essential amino acids, vitamins, other organic compounds, trace minerals, and inorganic salts. Medium 171PRF is a phenol red-free version of Medium 171. These media do not contain antibiotics, antimycotics, hormones, growth factors, or proteins. These media are HEPES and bicarbonate buffered and are designed for use in an incubator with an atmosphere of 5% CO₂/95% air. To support plating and long-term proliferation of normal human mammary epithelial cells, these media must be supplemented with Mammary Epithelial Growth Supplement (MEGS, cat. no S-015-5).

Intended Use

Medium 171 is intended for use in the routine culture of normal human mammary epithelial cells. Medium 171PRF is intended for use by investigators who wish to culture normal human mammary epithelial cells in the absence of phenol red. When supplemented with MEGS, these media will support the plating and proliferation of normal human mammary epithelial cells at densities between 2.5×10^3 cells/cm² and 8×10^4 cells/cm². **This product is for research use only. Not for use in animals, humans, or diagnostic procedures.**

Caution: If handled improperly, some components of this product may present a health hazard. Take appropriate precautions when handling this product, including the wearing of protective clothing and eyewear. Dispose of properly.

Storage and Stability

Medium 171 and Medium 171PRF are stored at 4° C in our facility and are shipped at ambient temperature. Upon receipt, these media should be stored at 4° C and should not be frozen. **Protect from light.** Several components of these tissue culture media are light-labile, and we recommend that the media not be exposed to light for lengthy periods of time. If the media are warmed prior to use, do not exceed 37° C. When stored in the dark at 4° C, the product is stable until the expiration date on the label.

Preparation of Supplemented Medium 171

1. Thaw one bottle of MEGS. Take one bottle of Medium from cold storage. Make sure that the caps of the vessels are tight.
2. Gently swirl the bottle of supplement. Avoid splashing the supplement into the cap of the bottle or causing the supplement to foam.
3. Wipe the outside of the containers with a disinfecting solution such as 70% ethanol or isopropanol.
4. Using sterile technique in a laminar flow culture hood, transfer the entire contents of the bottle of supplement to the bottle of Medium.
5. Tightly cap the bottle of supplemented medium and swirl the contents to ensure a homogeneous solution. Avoid causing the medium to foam.

Storage and Stability of Supplemented Medium 171

Once Medium 171 or Medium 171PRF has been supplemented with MEGS, the supplemented medium should be stored in the dark at 4° C and should not be frozen. When stored in the dark at 4° C, the supplemented medium is stable for 1 month.

Selected References

The Medium 171 formulation is based on medium MCDB 170, with modifications.

Hammond SL, Ham RG, Stampfer MR; PNAS 81:5435-5439, 1984

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Fig. 4 continued

Cascade Biologics™
invitrogen cell culture

GIBCO®
invitrogen cell culture

MEGS Mammary Epithelial Growth Supplement

Cat. no. S-015-5
5 ml

Product Description

Mammary Epithelial Growth Supplement (MEGS) is a sterile, concentrated (100X) solution intended for use as one component in a complete culture environment for the growth of normal human mammary epithelial cells. Each 5 ml bottle of MEGS contains all of the growth factors, hormones, and tissue extracts necessary for the culture of normal human mammary epithelial cells and is the correct amount of supplement for a 500 ml bottle of Medium 171 or Medium 171PRF. MEGS is an ionically-balanced supplement containing bovine pituitary extract (BPE), bovine insulin, hydrocortisone and recombinant human epidermal growth factor. When a 500 ml bottle of Medium 171 or Medium 171PRF is supplemented with MEGS, the final concentrations of the components in the supplemented medium are: BPE, 0.4% v/v; bovine insulin, 5 µg/ml; hydrocortisone, 0.5 µg/ml; and recombinant human epidermal growth factor, 3 ng/ml.

Intended Use

MEGS is intended for use in conjunction with Medium 171 or Medium 171PRF for the routine serum-free culture of normal human mammary epithelial cells. ***This product is for research use only. Not for use in animals, humans, or diagnostic procedures.***

Caution: If handled improperly, some components of this product may present a health hazard. Take appropriate precautions when handling this product, including the wearing of protective clothing and eyewear. Dispose of properly.

Limited Use Label License No. 5: Invitrogen Technology

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For products that are subject to multiple limited use label licenses, the terms of the most restrictive limited use label license shall control. Life Technologies Corporation will not assert a claim against the buyer of infringement of patents owned or controlled by Life Technologies Corporation which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Life Technologies is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500. Email: outlicensing@invitrogen.com.

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MAN0001585

Storage and Stability

MEGS is stored at -20°C at our facility and is shipped on dry ice. Upon receipt, the product should be stored at -20°C in a freezer that is not self-defrosting. When stored at -20°C , the product is stable until the expiration date shown on the label.

After long-term storage at -20°C , MEGS may contain a small amount of precipitate. This precipitate is formed from cold-insoluble material in the BPE component of the MEGS and will not affect the performance of the product.

Thawing

To thaw, place the product in a 37°C water bath or overnight at 4°C . If thawed in a water bath, do not leave the product at 37°C after the product has thawed. For instructions on adding MEGS to Medium 171, please refer to the instructions that accompany the basal medium.

Selected References

The MEGS formulation is based on published supplementation of medium MCDB 170, with modifications.

Hammond SL, Ham RG, Stampfer MR; PNAS 81:5435-5439, 1984.

Fig. 5**PTT6 Medium ingredients list**

| Medium composition List | Company Name | Catalogue Number |
|-------------------------|--------------|------------------|
|-------------------------|--------------|------------------|

Basic media

| | | |
|---|-------------------|---------|
| DMEM (also referred to herein as PTT6-basal medium) | Lonza | 12-604F |
| DMEM/F12 | Lonza | 12-719F |
| M171 | Life technologies | M171500 |

Serum

| | | |
|--------------------|---------------|---------|
| Fetal Bovine Serum | GE Healthcare | A15-151 |
|--------------------|---------------|---------|

Antibiotic

| | | |
|--|-------|---------|
| Penicillin-Streptomycin-Amphotericin B | Lonza | 17-745E |
|--|-------|---------|

Supplements

| | | |
|--|-------------------|-----------|
| Adenine (optional) | Sigma | A8626-25G |
| Hydrocortisone (optional) | Sigma | H-0888 |
| Epidermal Growth Factor | Millipore | GF-144 |
| T3 (3,3',5-Triiodo-L-thyronine sodium salt) (optional) | Sigma | 200-223-5 |
| Recombinant Human Insulin AOF | Life Technologies | A11382IJ |

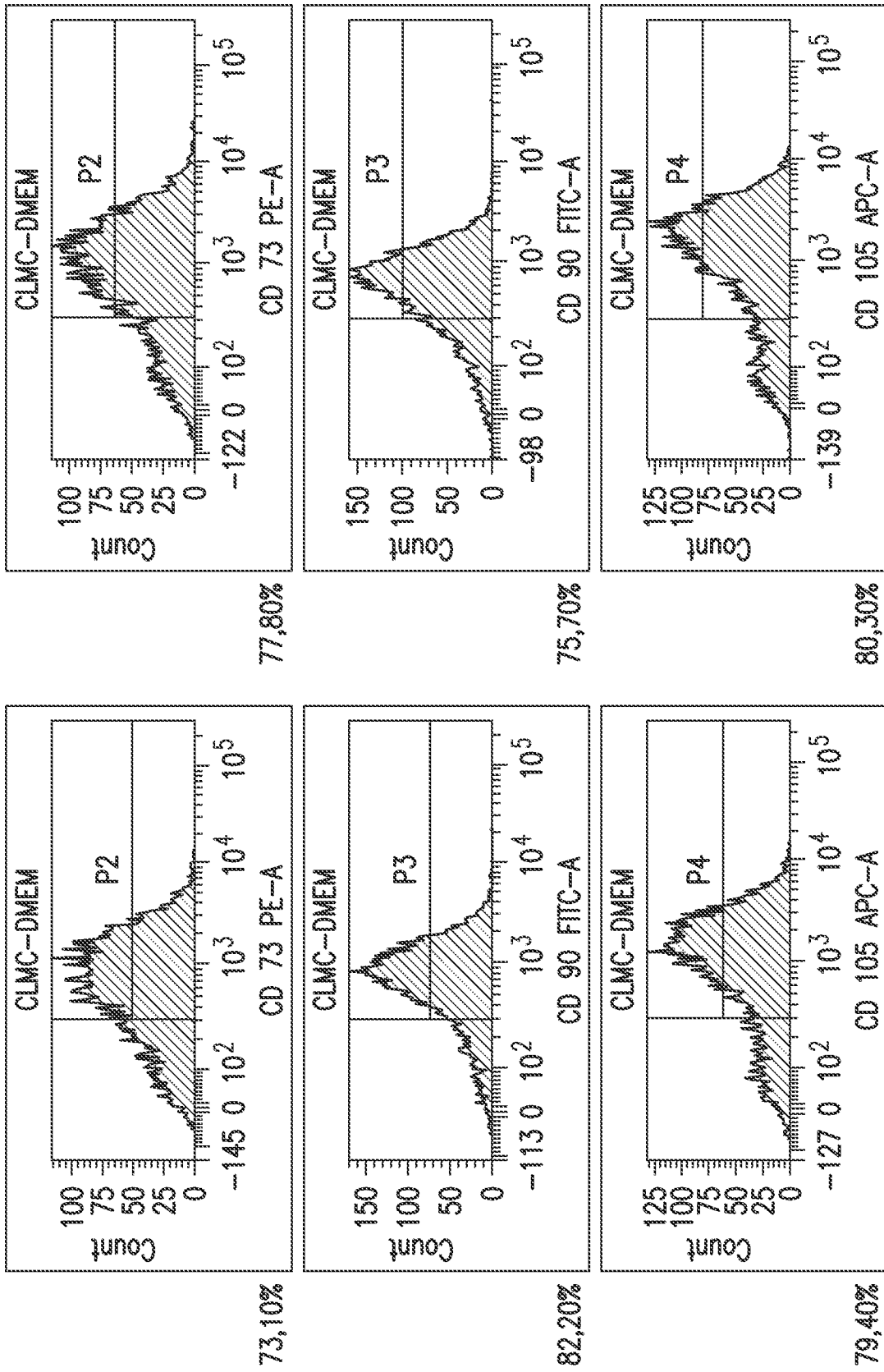


FIG. 6A

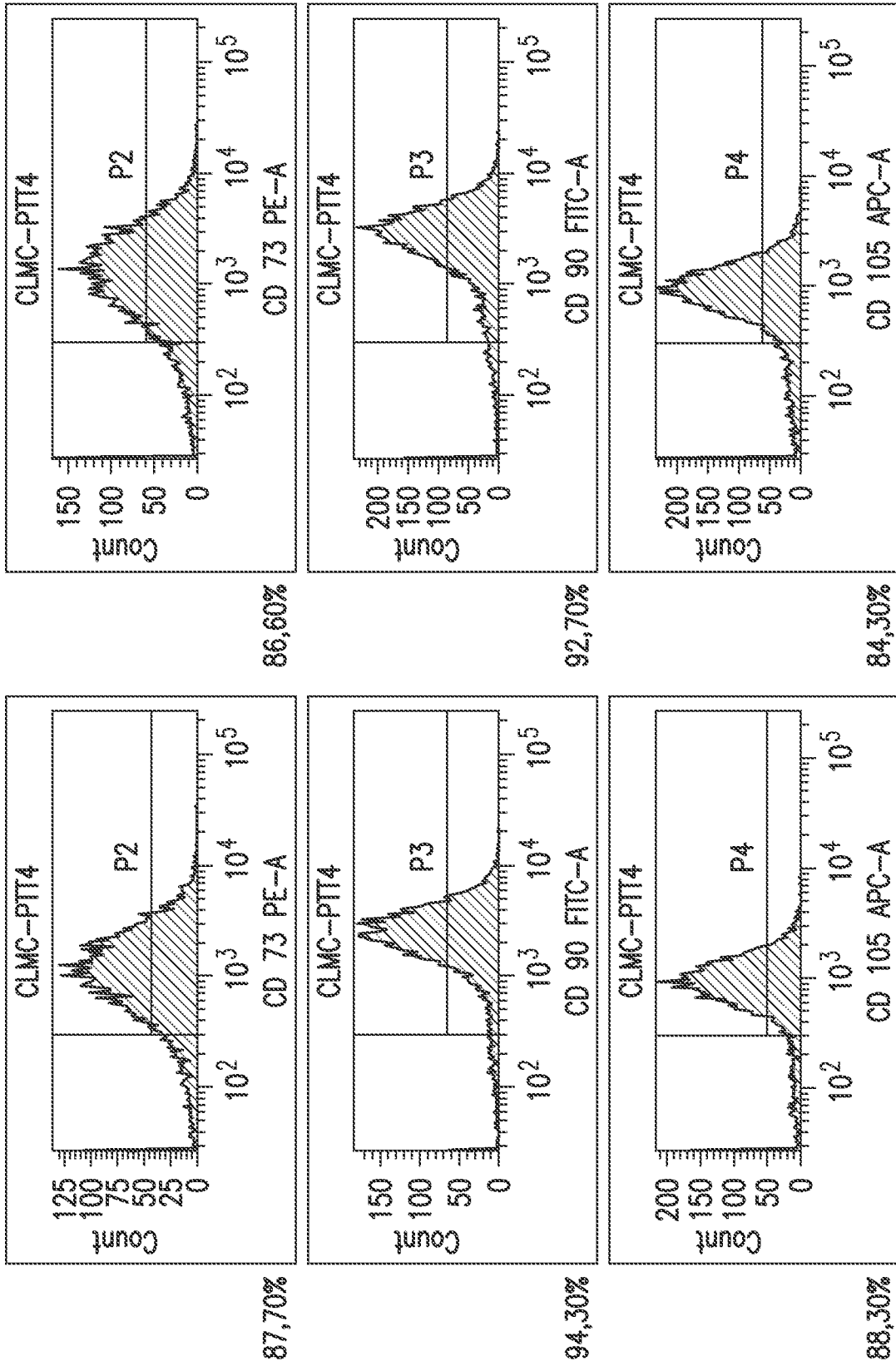


FIG. 6B

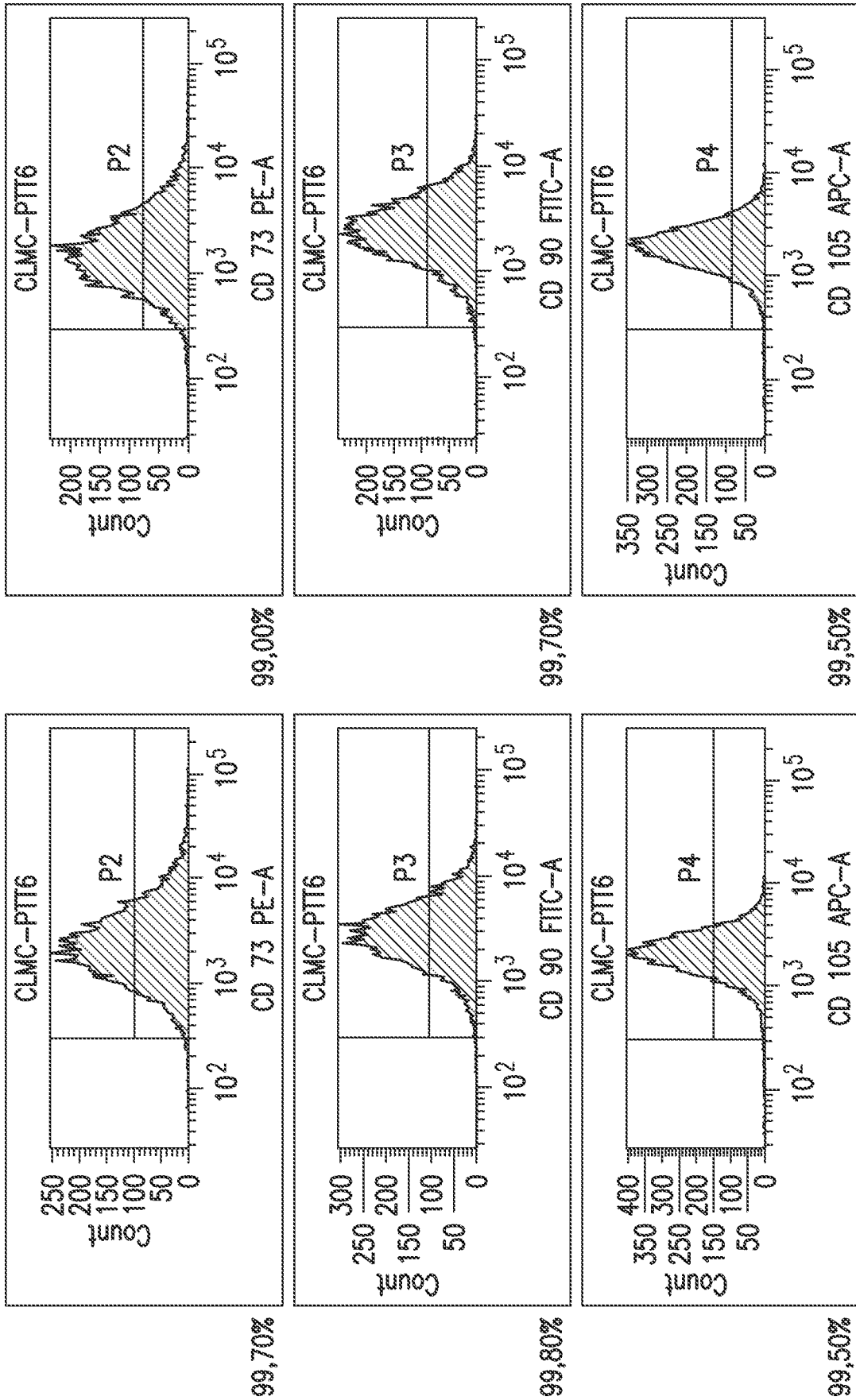
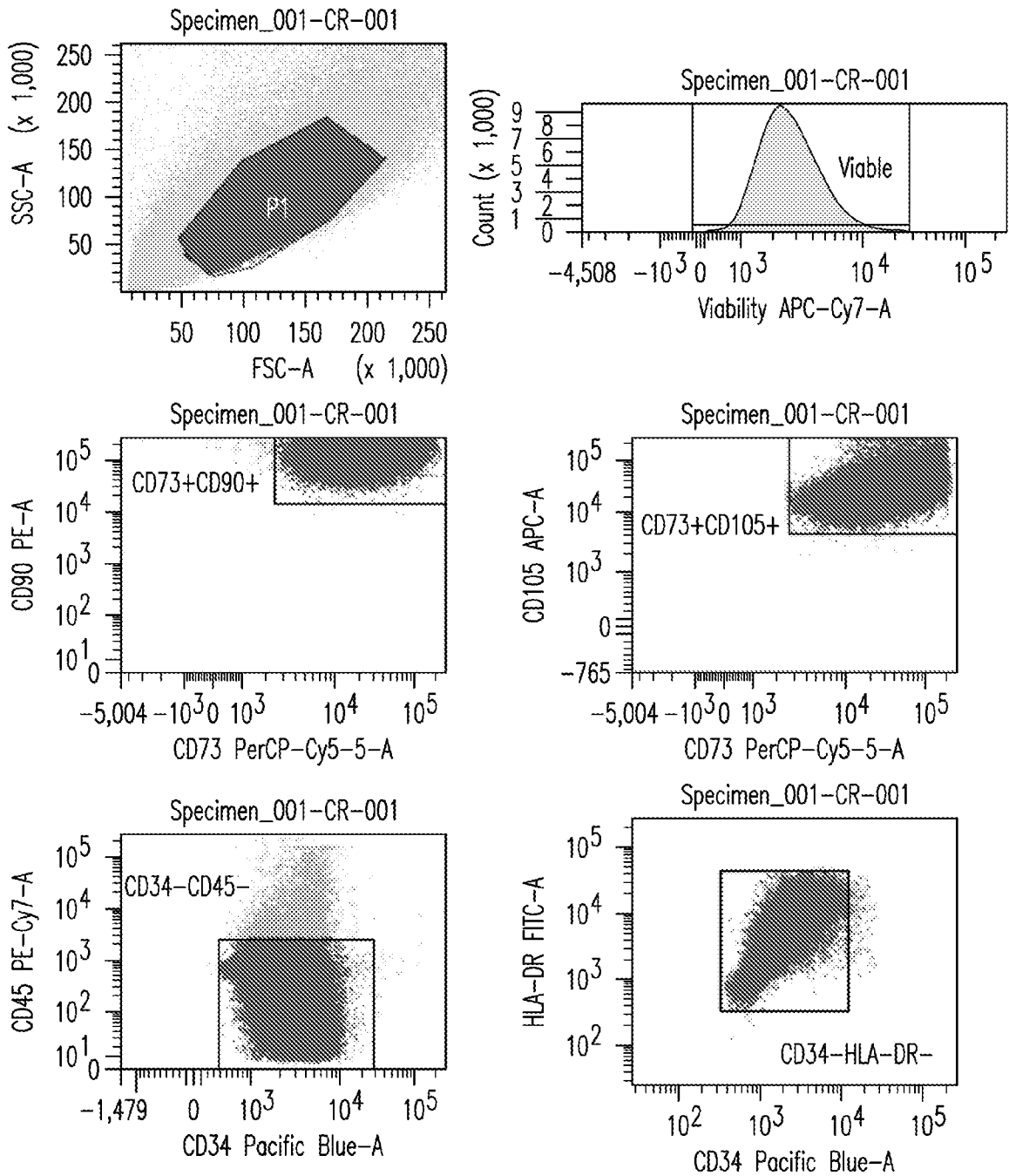


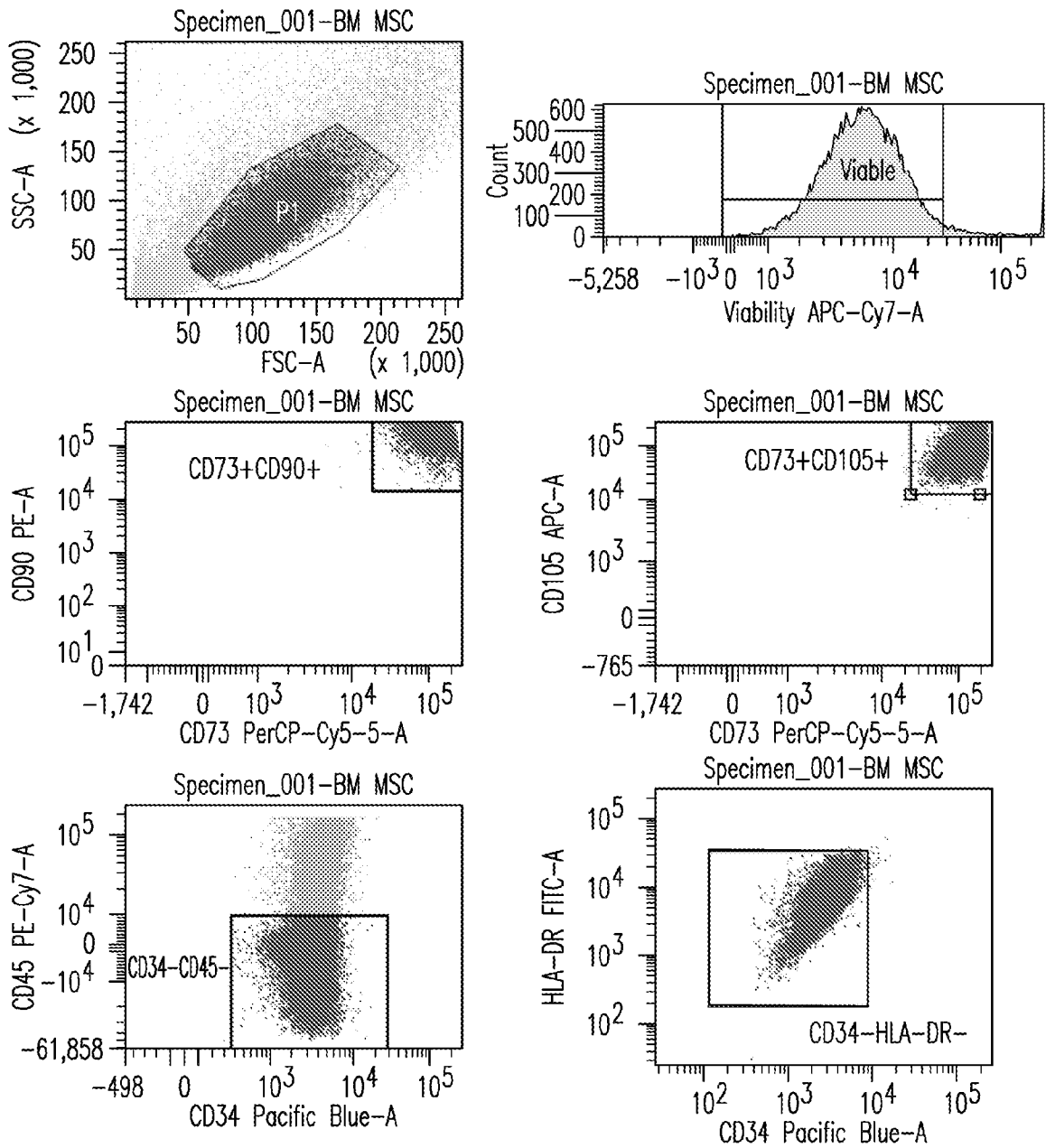
FIG. 6C

BD FACSDiva 8.0.1



| Tube: CR-001 | | | |
|--------------|---------|---------|--------|
| Population | #Events | %Parent | %Total |
| All Events | 540.988 | #### | 100.0 |
| P1 | 425.093 | 78.6 | 78.6 |
| Viable | 414.643 | 97.5 | 76.6 |
| CD73+CD90+ | 414.554 | 100.0 | 76.6 |
| CD73+CD105+ | 414.490 | 100.0 | 76.6 |
| CD34-CD45- | 411.206 | 99.2 | 76.0 |
| CD34-HLA-DR | 411.061 | 100.0 | 76.0 |

FIG.7A



| | | | |
|------------------|--------------|---------|--------|
| Experiment Name: | MSC 062216 | | |
| Specimen Name: | Specimen_001 | | |
| Tube Name: | BM MSC | | |
| Population | #Events | %Parent | |
| CD34-HLA-DR- | 21.128 | 99.9 | |
| Population | #Events | %Parent | %Total |
| All Events | 77.022 | ### | 100.0 |
| P1 | 35.780 | 46.5 | 46.5 |
| Viable | 33.741 | 94.3 | 43.8 |
| CD73+CD90+ | 33.729 | 100.0 | 43.8 |
| CD73+CD105+ | 33.710 | 99.9 | 43.8 |
| CD34-CD45- | 21.155 | 62.8 | 27.5 |
| CD34-HLA-DR | 21.128 | 99.9 | 27.4 |

FIG.7B

Figure 8

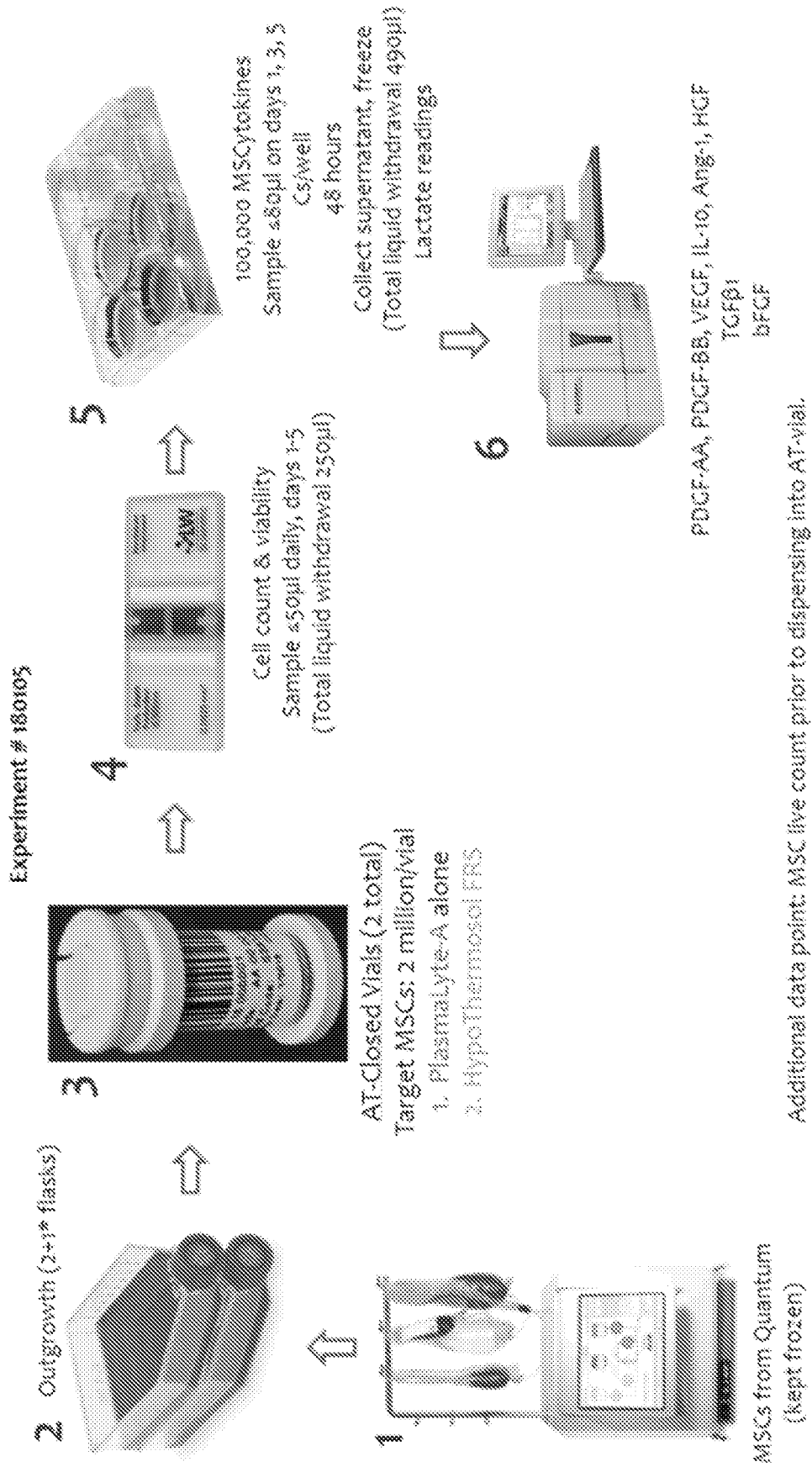
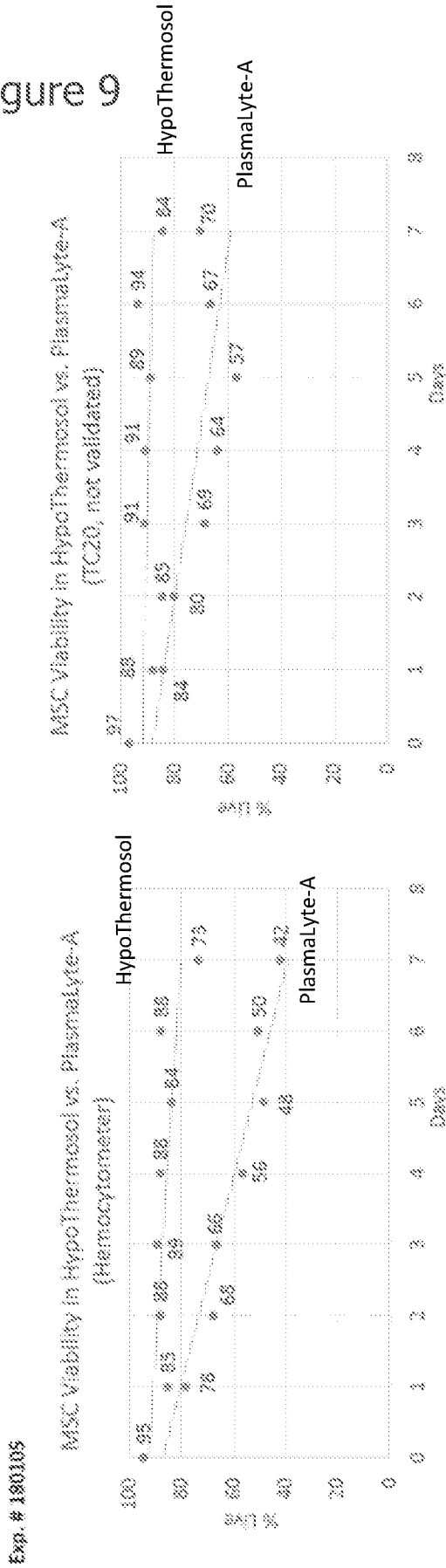


Figure 9

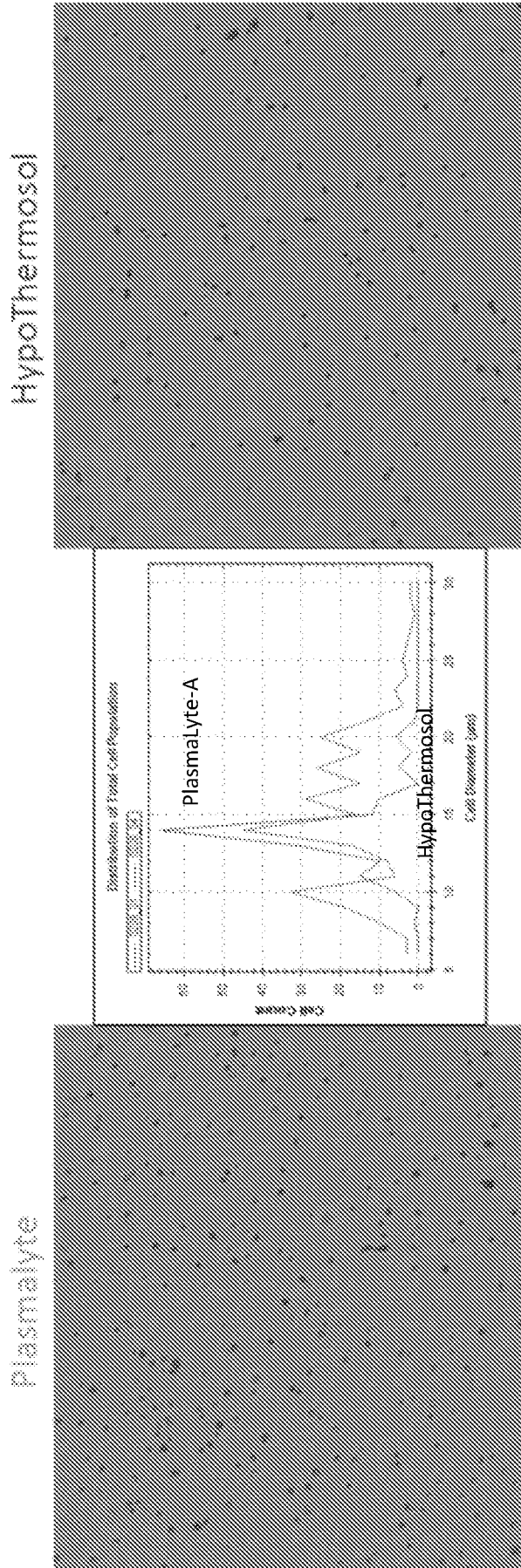


| Total cell counts | Hemocytometer | Std. dev. | % std. dev. | TC20 | Std. dev. | % std. dev. |
|-------------------|---------------|-----------|-------------|-----------|-----------|-------------|
| HypoThermosol | 802,500 | 119,155 | 14.8 | 758,357 | 104,994 | 13.8 |
| Plasmalyte | 1,608,572 | 213,902 | 13.3 | 1,301,571 | 208,991 | 16.1 |

- HypoThermosol noticeably supports membrane integrity and presumably survival over a week timespan (6 days).
- All counts based on duplicate readings that are within 10% of one another. Following SOP CR D2.600.1.
- During counting, cells stored in HypoThermosol were noticeably smaller with smooth and defined edges. By contrast, cells in Plasmalyte appeared of a range of sizes.

Figure 10

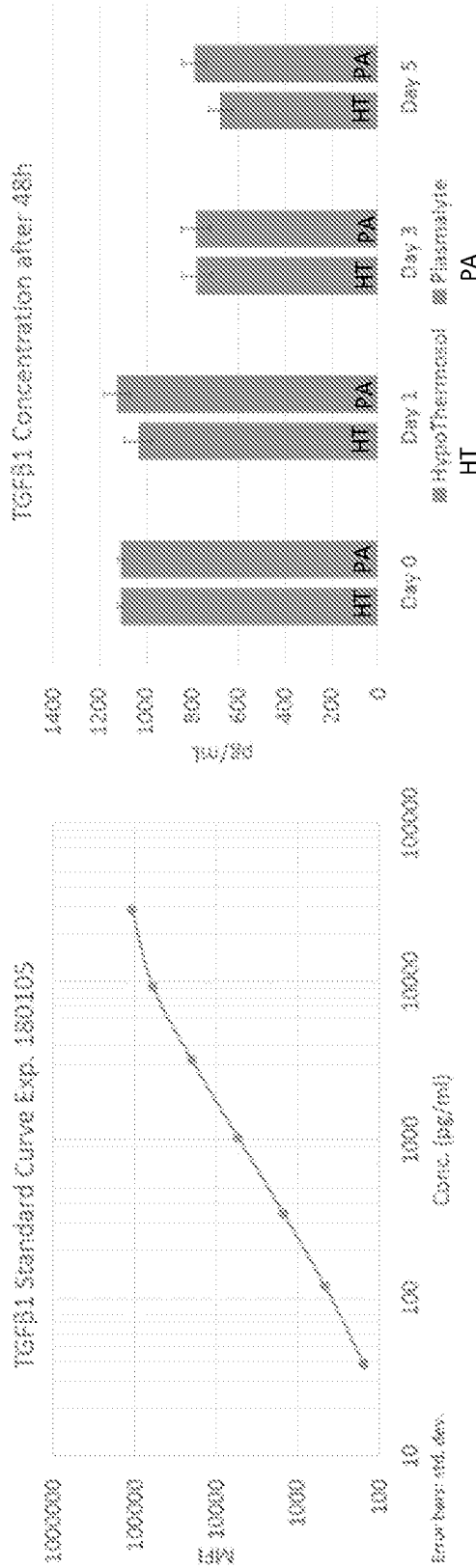
Day 3 TC20 images



- TC20 confirms that cells kept in HypoThermosol are of a narrow diameter range (in this example, comparison after 3 days of storage).

Figure 11

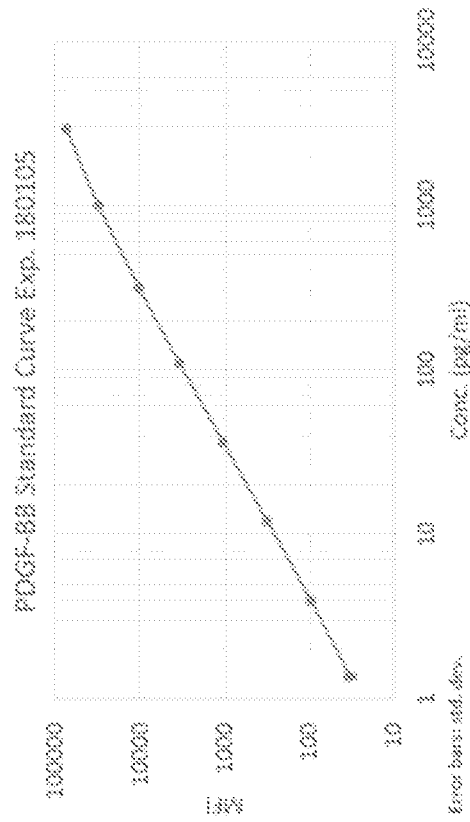
TGFβ1 Concentration after 48h in Media from MSCs stored in HypoThermosol or Plasmalyte



| Days of storage | 2 | 137 | 140 | 143 | 332 |
|-----------------|-----------|----------|----------|-----------|-----------|
| Conc. (pg/ml) | 1046 ± 34 | 830 ± 33 | 952 ± 22 | 1154 ± 36 | 1046 ± 34 |

Figure 12

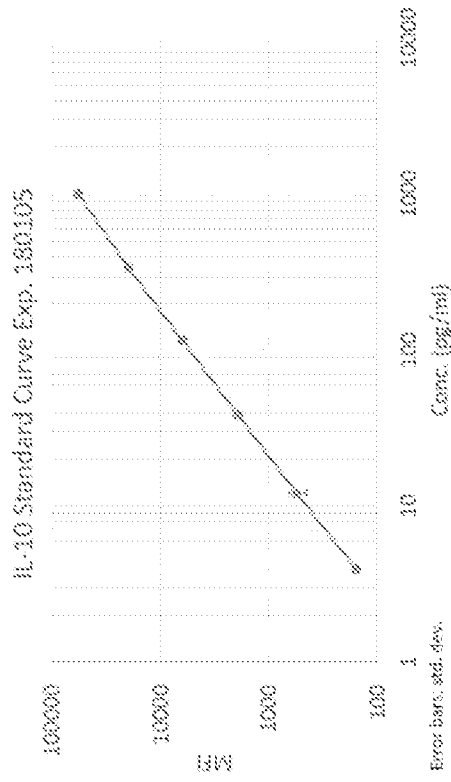
PDGF-BB Concentration after 48h in Media from MSCs stored in HypoThermosol or PlasmaLyte



PDGF-BB not detected in any samples.

Figure 13

IL-10 Concentration after 48h in Media from MSCs stored in HypoThermosol or PlasmaLyte



IL-10 not detected in any samples.

Figure 14

VEGF Concentration after 48h in Media from MSCs stored in HypoThermosol or PlasmaLyte

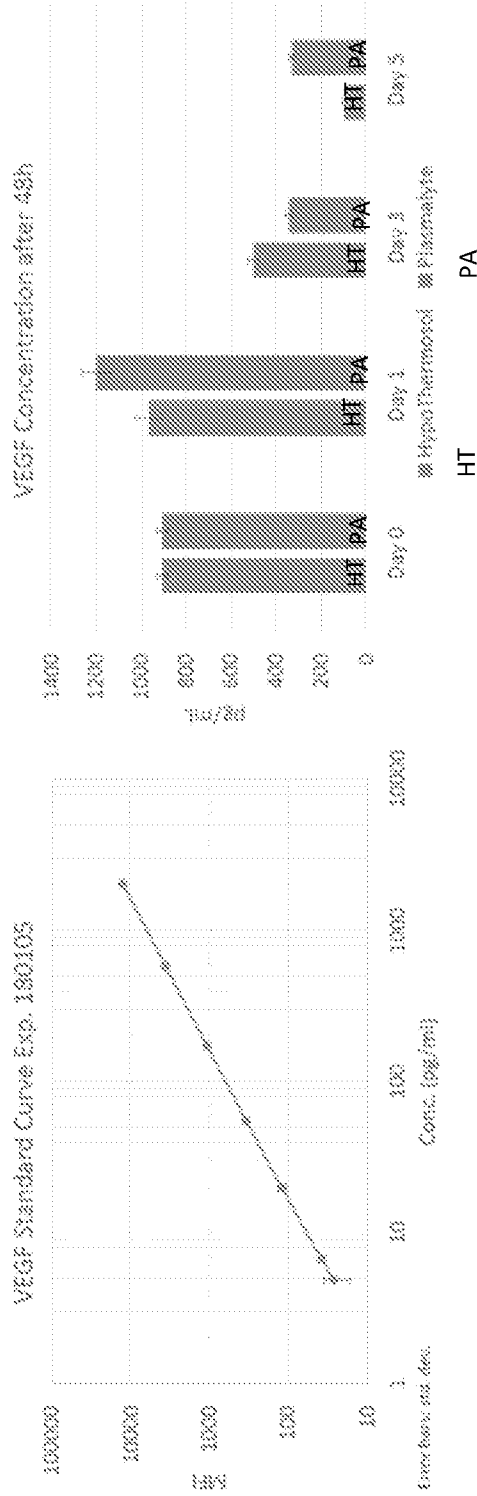


Figure 15

PDGF-AA Concentration after 48h in Media from MSCs stored in HypoThermosol or PlasmaLyte

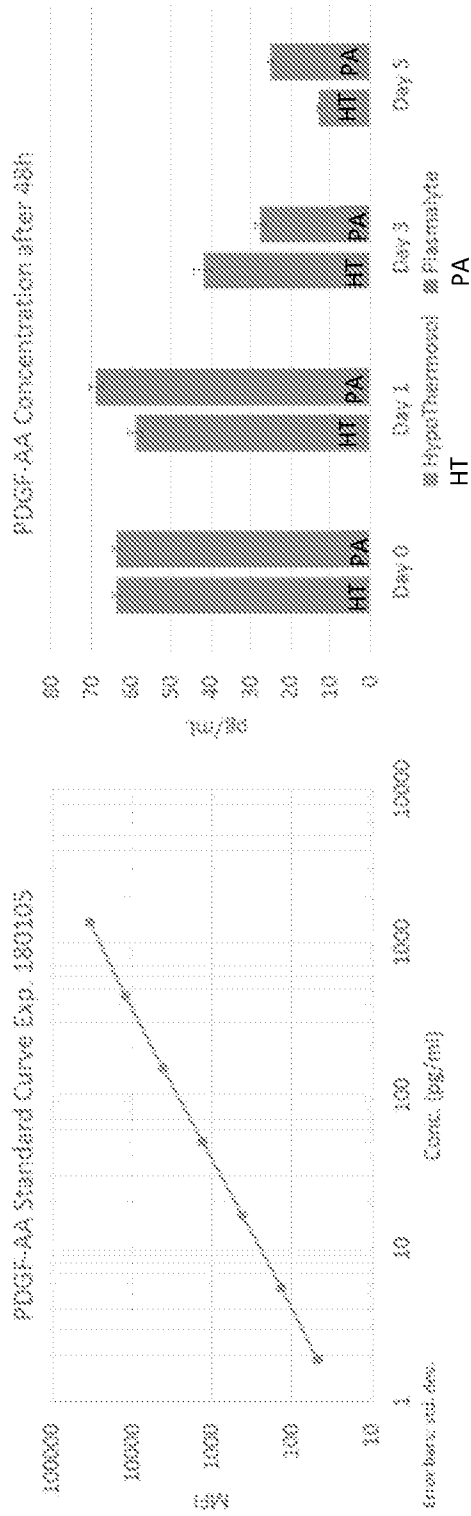


Figure 16

Ang-1 Concentration after 48h in Media from MSCs stored in HypoThermosol or PlasmaLyte

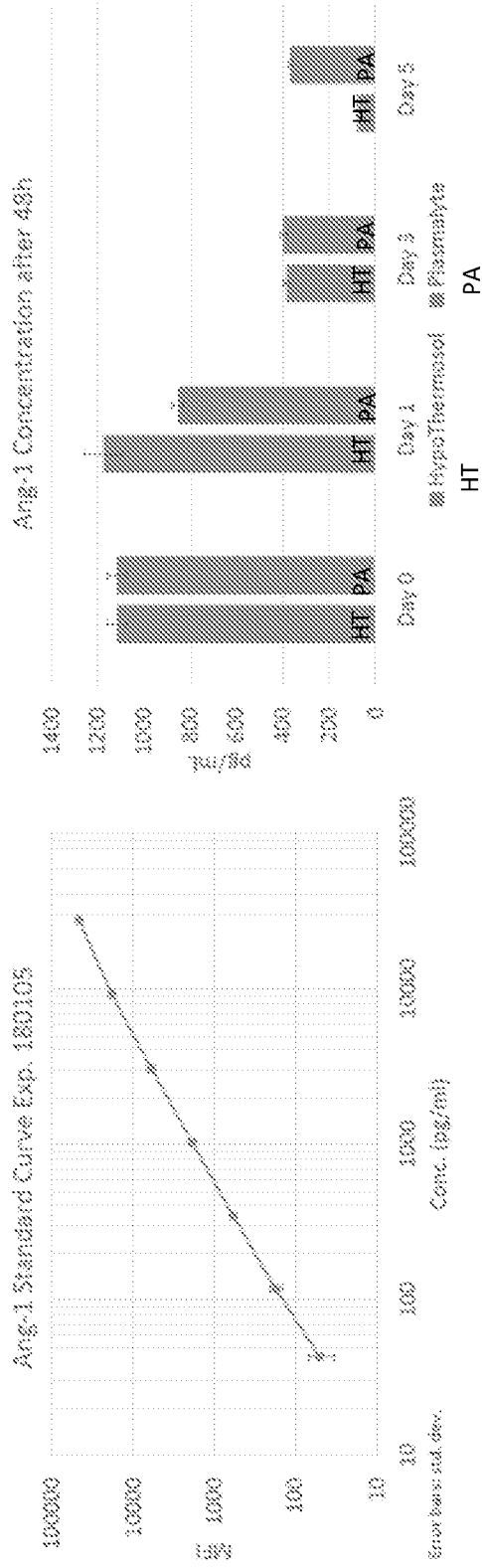


Figure 17

HGF Concentration after 48h in Media from MSCs stored in HypoThermosol or PlasmaLyte

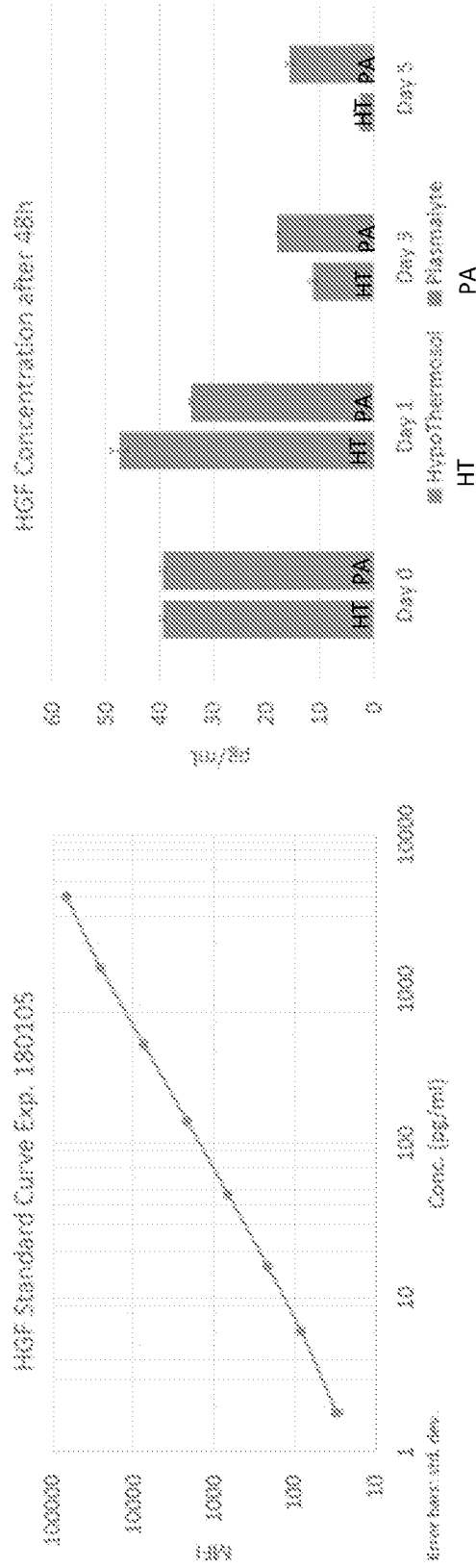


Figure 18

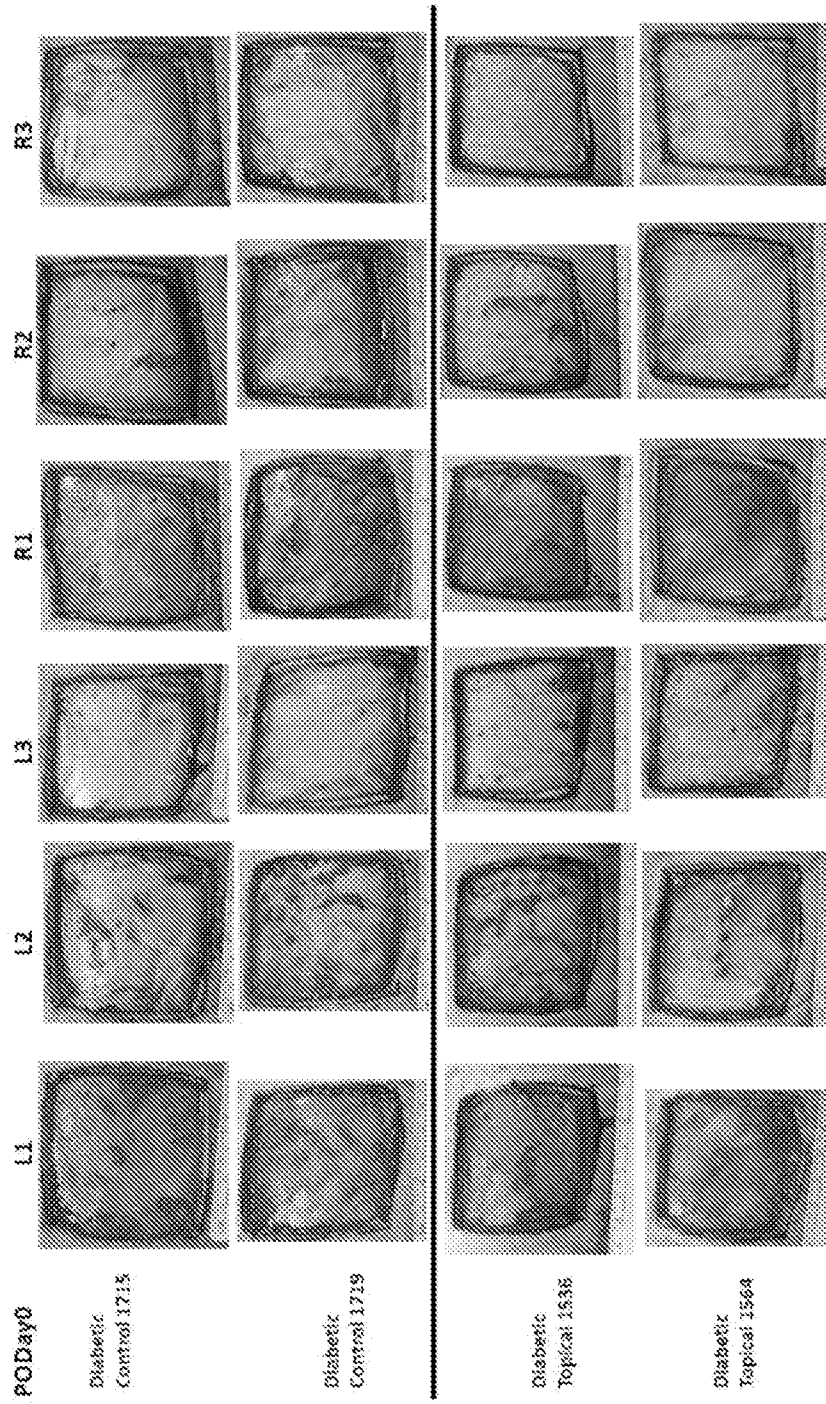


Figure 18 cont'

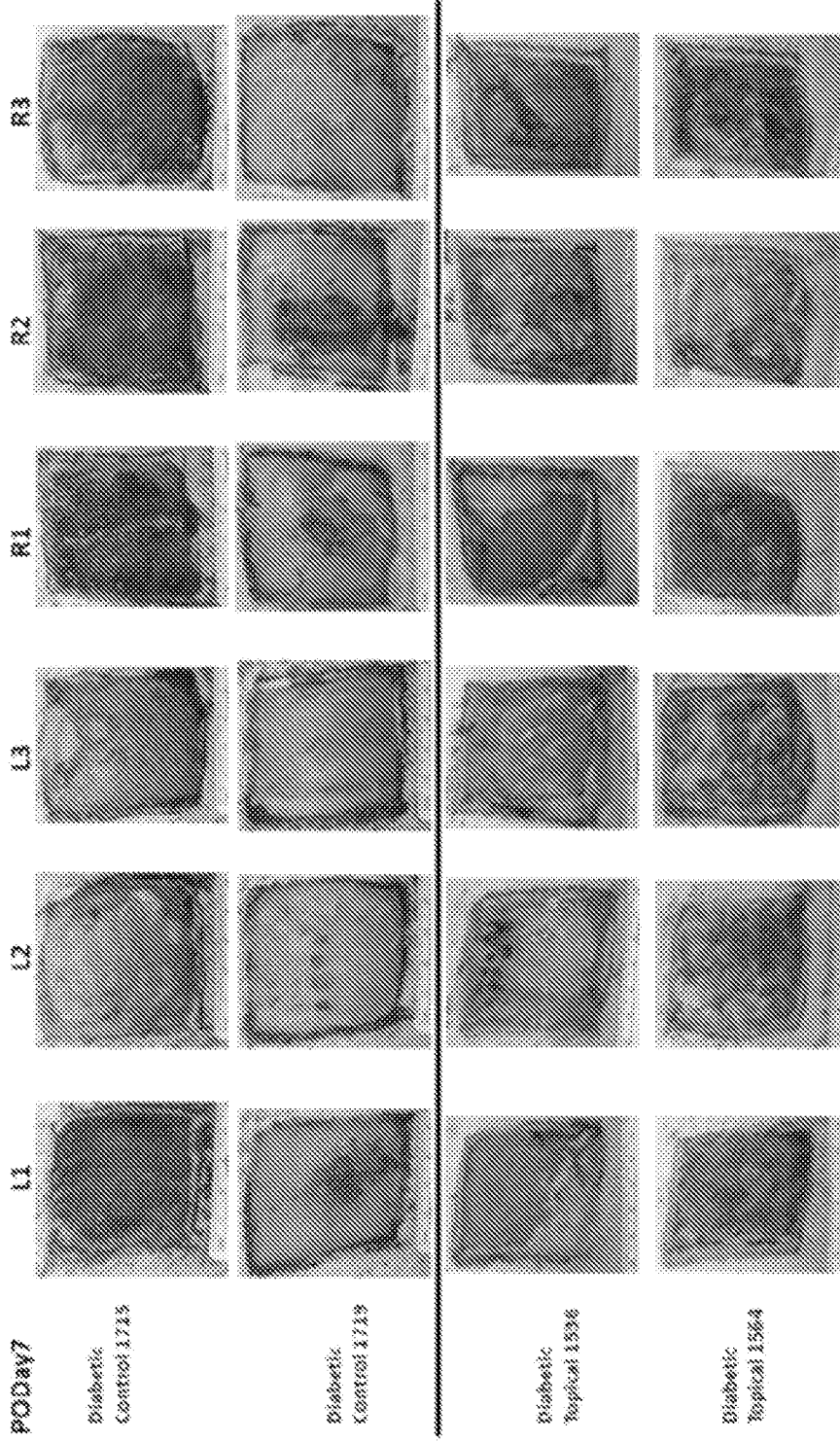


Figure 18 cont'

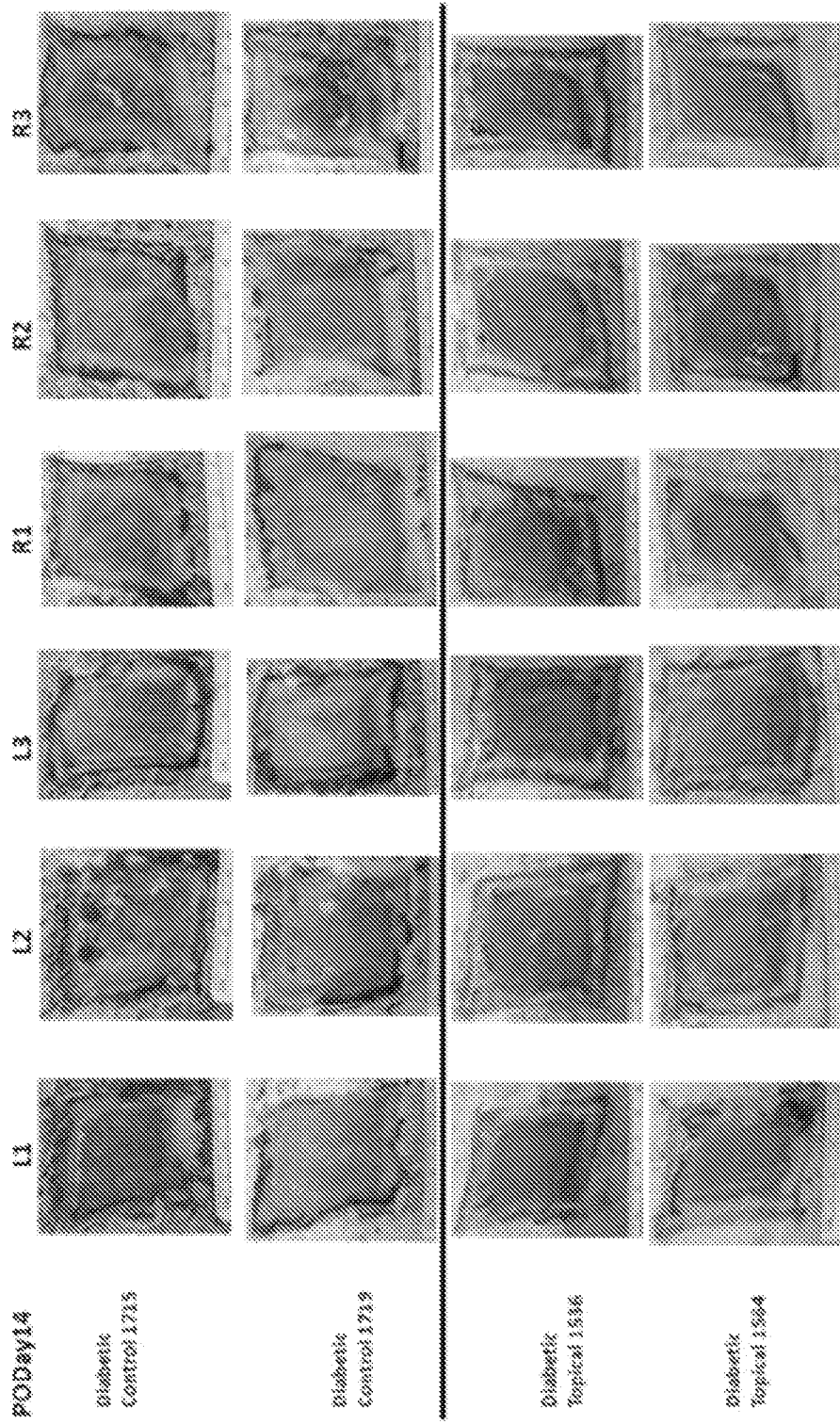


Figure 18 cont'

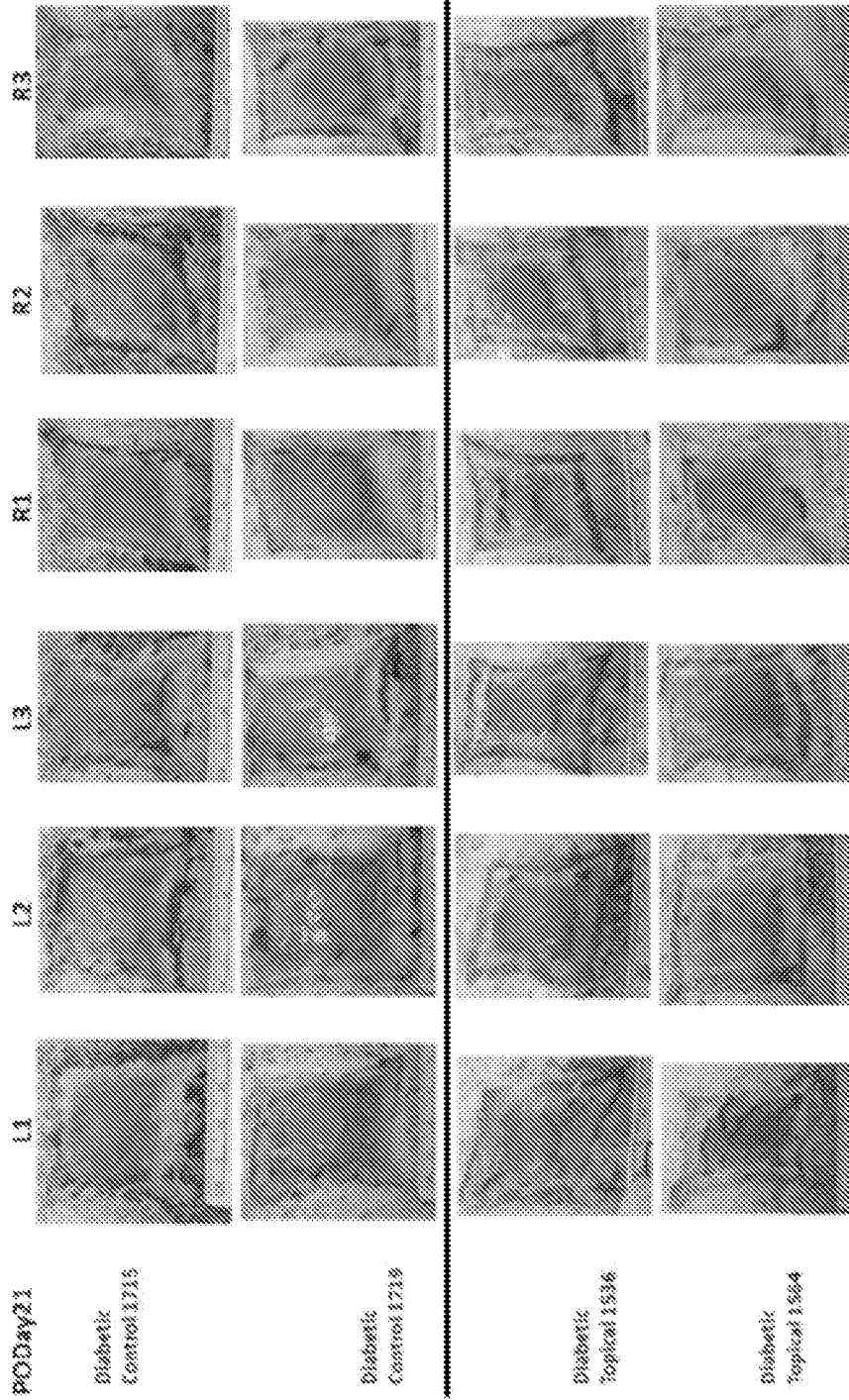
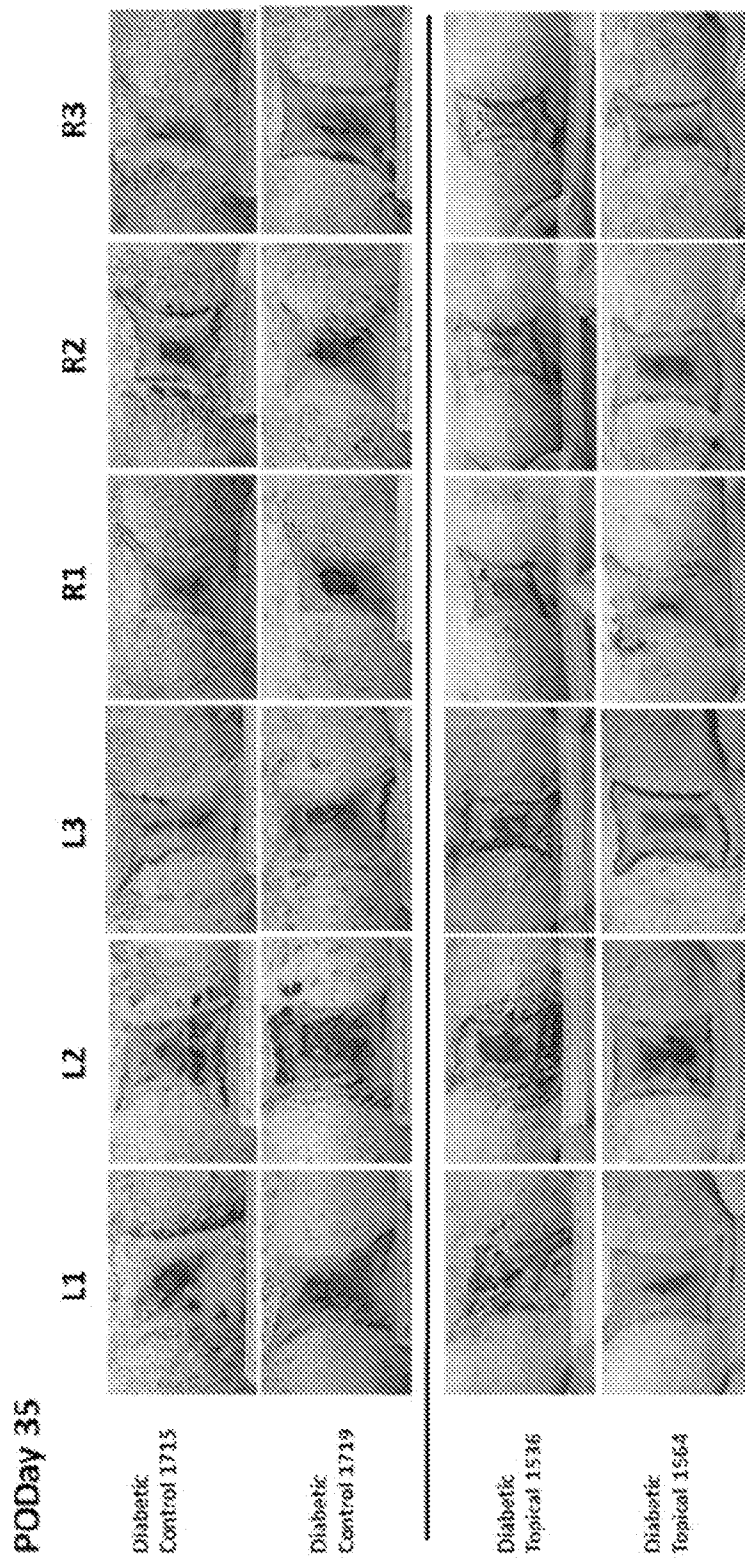


Figure 18 cont'



Certificate of Analysis

www.tocris.com
Product Name: Trolox

Catalog No.: 6002

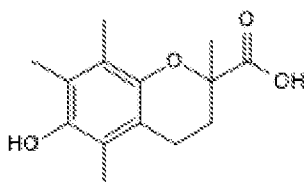
Batch No.: 1

CAS Number: 53188-07-1

IUPAC Name: 3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-carboxylic acid

1. PHYSICAL AND CHEMICAL PROPERTIES

| | |
|-----------------------------------|---|
| Batch Molecular Formula: | C ₁₄ H ₁₈ O ₄ ·½H ₂ O |
| Batch Molecular Weight: | 259.3 |
| Physical Appearance: | Beige solid |
| Solubility: | DMSO to 100 mM ethanol to 100 mM |
| Storage: | Store at +4°C |
| Batch Molecular Structure: | |



2. ANALYTICAL DATA

| | | | |
|---------------------------|---------------------------|-------|------|
| HPLC: | Shows 99.7% purity | | |
| ¹H NMR: | Consistent with structure | | |
| Mass Spectrum: | Consistent with structure | | |
| Microanalysis: | Carbon Hydrogen Nitrogen | | |
| | Theoretical | 64.85 | 7.39 |
| | Found | 64.72 | 7.58 |

Caution - Not Fully Tested • Research Use Only • Not For Human or Veterinary Use

bio-techne.com
 info@bio-techne.com
 techsupport@bio-techne.com

North America
 Tel: (800) 343 7475

China
 info.cn@bio-techne.com
 Tel: +86 (21) 52380373

Europe Middle East Africa
 Tel: +44 (0)1235 529449

Rest of World
www.tocris.com/distributors
 Tel: +1 612 379 2956

Product Information

Product Name: Trolox**Catalog No.:** 6002**Batch No.:** 1

CAS Number: 53188-07-1

IUPAC Name: 3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-carboxylic acid

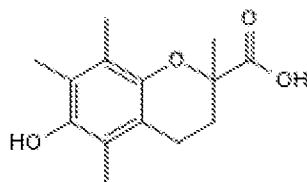
Description:

Antioxidant vitamin E derivative; suppresses osteoclast formation and inhibits IL-1-induced osteoclast bone loss. Cell-permeable and water soluble.

Physical and Chemical Properties:Batch Molecular Formula: C₁₄H₁₈O₄·½H₂O

Batch Molecular Weight: 259.3

Physical Appearance: Beige solid

Minimum Purity: >98%**Batch Molecular Structure:****Storage:** Store at +4°C**Solubility & Usage Info:**

DMSO to 100 mM

ethanol to 100 mM

Stability and Solubility Advice:

Some solutions can be difficult to obtain and can be encouraged by rapid stirring, sonication or gentle warming (in a 45-60°C water bath).

Information concerning product stability, particularly in solution, has rarely been reported and in most cases we can only offer a general guide. Our standard recommendations are:

SOLIDS: Provided storage is as stated on the product label and the vial is kept tightly sealed, the product can be stored for up to 6 months from date of receipt.

SOLUTIONS: We recommend that stock solutions, once prepared, are stored aliquoted in tightly sealed vials at -20°C or below and used within 1 month. Wherever possible solutions should be made up and used on the same day.

References:

Lee *et al* (2009) Trolox prevents osteoclastogenesis by suppressing RANKL expression and signaling. *J.Biol.Chem.* **284** 13725. PMID: 19299513.

Giulivi *et al* (1993) Inhibition of protein radical reactions of ferrylmyoglobin by the water-soluble analog of vitamin E, Trolox C. *Arch.Biochem.Biophys.* **303** 152. PMID: 8489259.

Caution - Not Fully Tested • Research Use Only • Not For Human or Veterinary Use

bio-techne.com

info@bio-techne.com

techsupport@bio-techne.com

North America

Tel: (800) 343 7475

China

info.cn@bio-techne.com

Tel: +86 (21) 52380373

Europe Middle East Africa

Tel: +44 (0)1235 529449

Rest of World

www.tocris.com/distributors

Tel: +1 612 379 2956

SIGMA-ALDRICH® Figure 20

sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA

Website: www.sigmaaldrich.com

Email USA: techserv@sial.com

Outside USA: eurtechserv@sial.com

Product Specification

Product Name:

Sodium chloride - PharmaGrade, USP, Manufactured under appropriate GMP controls for pharma or biopharmaceutical production.

Product Number: RES0926S-A7

CAS Number: 7647-14-5

Formula: NaCl

Formula Weight: 58.44 g/mol

| TEST | Specification |
|--|----------------|
| Appearance (Color) | White |
| Appearance (Form) | Powder |
| Appearance of Solution (Turbid 20% aqueous) | Clear |
| Appearance of Solution (Color) 20% aqueous | Colorless |
| Heavy Metals (per USP) | ≤ 5 ppm |
| Identification | Pass |
| Iodides | Pass |
| Iron (Fe) | ≤ 2.0 µG/g |
| Limit of Bromides | ≤ 0.010 % |
| Limit of Phosphate | ≤ 0.0025 % |
| Limit of Potassium | ≤ 0.05 % |
| Loss on Drying (USP) | ≤ 0.5 % |
| Acidity or Alkalinity | Pass |
| Aluminum (Al) | ≤ 0.2 µG/g |
| Arsenic (As) | ≤ 1.0 µG/g |
| Assay | 99.0 - 100.5 % |
| Dried basis | |
| Barium (USP) | Pass |
| Chloride | Pass |
| Ferrocyanides | Pass |
| Magnesium/Alkaline Earth Metal | Pass |
| Nitrites | Pass |
| Sulfate (SO ₄) | ≤ 0.020 % |

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

SIGMA-ALDRICH® Figure 20 cont¹*sigma-aldrich.com*

3050 Spruce Street, Saint Louis, MO 63103, USA

Website: www.sigmaaldrich.comEmail USA: techserv@sial.comOutside USA: eurtechserv@sial.com

Product Specification

Product Name:

Sodium chloride - PharmaGrade, USP, Manufactured under appropriate GMP controls for pharma or biopharmaceutical production.

Product Number:**RES0926S-A7****CAS Number:**

7647-14-5

Formula:

NaCl

Formula Weight:

58.44 g/mol

TEST**Specification**

Specification: PRD.0.ZQ5.10000051146

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

SIGMA-ALDRICH® Figure 21

sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA

Website: www.sigmaaldrich.comEmail USA: techserv@sial.comOutside USA: eurtechserv@sial.com

Product Specification

Product Name:

Potassium phosphate monobasic - 99.99% trace metals basis

Product Number:

229806

CAS Number:

7778-77-0

MDL:

MFCD00011401

Formula:
H₂KO₄P
Formula Weight:

136.09 g/mol



| TEST | Specification |
|--|--------------------------|
| Appearance (Color) | Conforms to Requirements |
| White or Colorless | |
| Appearance (Form) | Powder or Crystals |
| Purity (Titration by NaOH) | 22.3 - 23.2 % |
| % P | |
| ICP Major Analysis | Confirmed |
| Confirms Potassium and Phosphorus Components | |
| Trace Metal Analysis | ≤ 150.0 ppm |
| Purity | Meets Requirements |
| 99.99% Based On Trace Metals Analysis | |

Specification: PRD.2.ZQ5.10000038396

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

SIGMA-ALDRICH® Figure 22

sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA

Website: www.sigmaaldrich.com

Email USA: techserv@sial.com

Outside USA: eurtechserv@sial.com

Product Specification

Product Name:

HEPES - PharmaGrade, Manufactured under appropriate controls for use as a raw material in pharma or biopharmaceutical production, suitable for cell culture

Product Number: **RES6003H-B7**

CAS Number: 7365-45-9

Formula: C₈H₁₈N₂O₄S

Formula Weight: 238.3 g/mol

| TEST | Specification |
|-------------------------|-----------------------|
| Appearance (Color) | White |
| Appearance (Form) | Free Flowing Powder |
| Infrared Spectrum | Conforms to Reference |
| Solubility (Turbidity) | Clear |
| 1.0M aqueous | |
| Solubility (Color) | Colorless |
| 1.0M aqueous | |
| Solubility (Form) | Solution |
| 1.0M aqueous | |
| Water (by Karl Fischer) | ≤ 0.5 % |
| Titration by NaOH | ≥ 99.5 % |
| Cytotoxicity | Pass |
| DNase - Nickase | Pass |
| DNase-Endo/Exonuclease | Pass |
| RNAse Detection | Pass |
| pH | 4.7 - 5.3 |
| 1.0% aqueous | |
| Endotoxin | ≤ 50 EU/g |
| Yeast and Mold | ≤ 100 CFU/g |
| Bioburden | ≤ 100 CFU/g |
| Protease Detection | Pass |
| Residue on Ignition | ≤ 0.1 % |
| Arsenic (As) | ≤ 3.0 ppm |
| Iron (Fe) | ≤ 5.0 ppm |
| Zinc (Zn) | ≤ 5.0 ppm |

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

SIGMA-ALDRICH® Figure 22 cont'

sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA

Website: www.sigmaaldrich.comEmail USA: techserv@sial.comOutside USA: eurtechserv@sial.com**Product Specification**

Product Name:

HEPES - PharmaGrade, Manufactured under appropriate controls for use as a raw material in pharma or biopharmaceutical production, suitable for cell culture

Product Number: **RES6003H-B7**

CAS Number: 7365-45-9

Formula: C₈H₁₈N₂O₄S

Formula Weight: 238.3 g/mol

| TEST | Specification |
|-------------------------------------|---------------|
| Nickel (Ni) | ≤ 5.0 ppm |
| Magnesium (Mg) | ≤ 5.0 ppm |
| Calcium (Ca) | ≤ 30.0 ppm |
| Lead (Pb) | ≤ 5.0 ppm |
| Bismuth (Bi) | ≤ 5.0 ppm |
| Lithium (Li) | ≤ 5.0 ppm |
| Molybdenum (Mo) | ≤ 5.0 ppm |
| Aluminum (Al) | ≤ 5.0 ppm |
| Antimony (Sb) | ≤ 5.0 ppm |
| Barium (Ba) | ≤ 5.0 ppm |
| Cobalt (Co) | ≤ 5.0 ppm |
| Chromium (Cr) | ≤ 5.0 ppm |
| Cadmium (Cd) | ≤ 5.0 ppm |
| Copper (Cu) | ≤ 5.0 ppm |
| Manganese (Mn) | ≤ 5.0 ppm |
| UV Absorbance 260nm 1.0M aqueous | ≤ 0.05 Abs UN |
| UV Absorbance 280nm 1.0M aqueous | ≤ 0.04 Abs UN |

Specification: PRD.0.ZQ5.10000050458

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Figure 23

Safety Data Sheet

1. Identification of the substance

Product Name: Sodium lactobionate

Synonyms:

Catalog number: QW-1395

Supplier: Combi-Blocks, Inc., 7949 Silverton Ave # 915, San Diego, CA 92126, USA. Tel: 858-635-8950

2. Hazards identification

No known hazard.

3. Composition/information on ingredients.

Ingredient name: Sodium lactobionate

CAS number: 27297-39-8

4. First aid measures

Skin contact: Immediately wash skin with copious amounts of water for at least 15 minutes while removing contaminated clothing and shoes. If irritation persists, seek medical attention.

Eye contact: Immediately wash skin with copious amounts of water for at least 15 minutes. Assure adequate flushing of the eyes by separating the eyelids with fingers. If irritation persists, seek medical attention.

Inhalation: Remove to fresh air. In severe cases or if symptoms persist, seek medical attention.

Ingestion: Wash out mouth with copious amounts of water for at least 15 minutes. Seek medical attention.

5. Fire fighting measures

In the event of a fire involving this material, alone or in combination with other materials, use dry powder or carbon dioxide extinguishers. Protective clothing and self-contained breathing apparatus should be worn.

6. Accidental release measures

Personal precautions: Wear suitable personal protective equipment which performs satisfactorily and meets local/state/national standards.

Respiratory precaution: Wear approved mask/respirator

Hand precaution: Wear suitable gloves/gauntlets

Skin protection: Wear suitable protective clothing

Eye protection: Wear suitable eye protection

Methods for cleaning up: Mix with sand or similar inert absorbent material, sweep up and keep in a tightly closed container for disposal. See section 12.

Environmental precautions: Do not allow material to enter drains or water courses.

7. Handling and storage

Handling: This product should be handled only by, or under the close supervision of, those properly qualified in the handling and use of potentially hazardous chemicals, who should take into account the fire, health and chemical hazard data given on this sheet.

Storage: Store in closed vessels.

8. Exposure Controls / Personal protection

Engineering Controls: Use only in a chemical fume hood.

Personal protective equipment: Wear laboratory clothing, chemical-resistant gloves and safety goggles.

General hygiene measures: Wash thoroughly after handling. Wash contaminated clothing before reuse.

Figure 23 cont'

9. Physical and chemical properties

| | |
|---------------------------|---|
| <i>Appearance:</i> | Not specified |
| <i>Boiling point:</i> | No data |
| <i>Melting point:</i> | No data |
| <i>Flash point:</i> | No data |
| <i>Density:</i> | No data |
| <i>Molecular formula:</i> | C ₁₂ H ₂₁ O ₁₂ .Na |
| <i>Molecular weight:</i> | 380.3 |

10. Stability and reactivity

| | |
|--|-----------------------------------|
| <i>Conditions to avoid:</i> | Heat, flames and sparks. |
| <i>Materials to avoid:</i> | Oxidizing agents. |
| <i>Possible hazardous combustion products:</i> | Carbon monoxide, nitrogen oxides. |

11. Toxicological information

| | |
|---|--|
| <i>Acute toxicity:</i> | No data available. |
| <i>Skin irritation/corrosion:</i> | No data available. |
| <i>Eye damage/irritation:</i> | No data available. |
| <i>Respiratory or skin sensitization:</i> | No data available. |
| <i>Germ cell mutagenicity:</i> | No data available. |
| <i>Carcinogenicity:</i> | No classification data on carcinogenic properties of this material is available from EPA, IARC, NTP, OSHA or ACGIH. |
| <i>Reproductive toxicity:</i> | No effects known. |
| <i>Specific target organ system toxicity - repeated exposure:</i> | No effects known. |
| <i>Specific target organ system toxicity - single exposure:</i> | No data available. |
| <i>Aspiration hazard:</i> | No data available. |
| <i>Additional information:</i> | To the best of our knowledge, the chemical, physical and toxicological properties of this substance have not been thoroughly investigated. |

12. Ecological information

| | |
|---|--|
| <i>Toxicity:</i> | No data available. |
| <i>Persistence and degradability:</i> | No data available. |
| <i>Bioaccumulative potential:</i> | No data available. |
| <i>Mobility in soil:</i> | No data available. |
| <i>Result of PBT and vPvB assessment:</i> | PBT: Not applicable. vPvB: Not applicable. |
| <i>Other adverse effects:</i> | No further relevant information available. |

13. Disposal consideration

Arrange disposal as special waste, by licensed disposal company, in consultation with local waste disposal authority, in accordance with national and regional regulations.

14. Transportation information

No known hazard for air and ground transportation.

15. Regulatory information

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302, or have known CAS numbers that exceed the threshold reporting levels established by SARA Title III, Section 313.

16. Other information

This MSDS is correct to the best of our knowledge at the date of publication but does not purport to be all inclusive and shall be used only as a guide. Combi-Blocks shall not be held liable for any injury or damage resulting from handling or from contact with the above product.

Figure 23 cont'

USA 15026 ARK2195B - Sucrose, Low Endotoxin

SEMA-ALDRICH

Figure 24

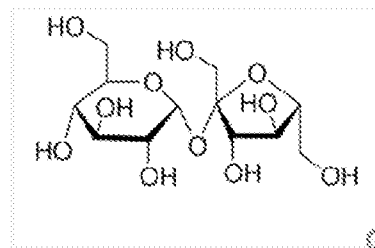
ARK2195B SEMA-ALDRICH

Sucrose, Low Endotoxin

PharmaGrade, USP/NF, Ph Eur, Manufactured under appropriate GMP controls for pharma or biopharmaceutical production.

Synonym: α-D-Glc (1→2)-β-D-Fru, α-D-Glucopyranosyl β-D-fructofuranoside, β-D-Fructofuranosyl-α-D-glucopyranoside, D(+)-Succharose, Sucrose, Sugar

50g



CAS Number 87-80-1 Empirical Formula (Hill Notation) C12H22O11 Molecular Weight 342.30

Beilstein Registry Number 80825 EC Number 209-234-9 MDL Number MFCD00006826

PubChem Substance ID 329730845

Properties

| | |
|-----------------------|--|
| Related Categories | Biochemicals and Reagents, Carbohydrates for Protein Stabilization, Proteins and Derivatives, Reagents for Protein Stabilization |
| biological source | plant |
| grade | Ph Eur USP/NF |
| InChI Key | C2MRCDWAGMRECN-UJGDZNRQSSA-N |
| product line | PharmaGrade |
| form | powder |
| impurities | Endotoxin; tested, Sulfites; tested |
| mp | 185-187 °C(lit.) |
| solubility | suitable for manufacturing use |
| Show Fewer Properties | |

Description

General description

PharmaGrade products are manufactured under appropriate controls for use as a raw material in pharma or biopharmaceutical production. For more information on the PharmaGrade Program, visit sigma-aldrich.com/usa-pharmagrade

Linkage

Quality Documentation on PharmaGrade Sucrose, Low Endotoxin

Price and Availability


| SKU-Pack Size | Availability | Price (USD) | Quantity |
|---------------|---|-------------|----------|
| ARK2195B-25KG | Estimated to ship on 05/05/19 | 5,596.70 | 0 |
| ARK2195B-1KG | Only 2 left in stock (based on the way) - F2CON | 376.70 | 0 |
| ARK2195B-10KG | Estimated to ship on 04/12/19 | 2,883.70 | 0 |
| ARK2195B-100G | Only 2 left in stock (based on the way) - F2CON | 292.00 | 0 |

BULK ORDERS?


ADD TO CART

Figure 24 cont'

Personalized Product Recommendations













S3929
Sucrose
meets USP testing specifications






16104
Sucrose
puriss., meets analytical specification of Ph. Eur., BP, NF

Customers Also Viewed

| | | | | |
|---|---|--|---|--|
|  S3929 Sucrose meets USP testing specifications |  16104 Sucrose puriss., meets analytical specification of Ph. Eur., BP, NF |  84097 Sucrose BioUltra, for molecular biology, ≥99.5% (HPLC) |  1.00892 Sucrose Sucrose low in endotoxins suitable for use as excipient EMPROVE® exp Ph Eur,BP,JP,NF, CAS 57-50-1, pH 7 (100 g/l, H ₂ O, 20 °C), low in endotoxins suitable for use as excipient EMPROVE® exp Ph Eur,BP,JP,NF |  S7903 Sucrose BioXtra, ≥99.5% (GC) |
|  S0389 Sucrose for molecular biology, ≥99.5% (GC) |  S9378 Sucrose ≥99.5% (GC) |  S1600000 Sucrose European Pharmacopoeia (EP) Reference Standard |  S1888 Sucrose BioReagent, suitable for cell culture, suitable for insect cell culture, ≥99.5% (GC) |  1.37048 D(+)-Glucose D(+)-Glucose anhydrous suitable for the biopharmaceutical production EMPROVE® bio Ph Eur,BP,USP, CAS 50-99-7, molar mass 180.16 g/mol., anhydrous suitable for the biopharmaceutical production EMPROVE® bio Ph Eur,BP,USP,ACS |

Recently Viewed

| | | | | |
|--|--|---|---|---|
|  L3376 Sodium lactobionate monohydrate 98.0-102.0% (EDTA titration) |  153516 Lactic acid 97% |  RES6003H-B7 RESIN PharmaGrade, Manufactured under appropriate controls for use as a raw material in pharma or biopharmaceutical production, suitable for cell culture | KH_2PO_4 229806 Potassium phosphate monobasic 99.99% trace metals basis | KH_2PO_4 RES20760-A7 Potassium phosphate monobasic PharmaGrade, NF, Manufactured under appropriate GMP controls for pharma or biopharmaceutical production. |
|--|--|---|---|---|

Safety Information

| | |
|-------------|---------------------------------|
| RIDADR | NONH for all modes of transport |
| WGK Germany | 2 |
| RTECS | WN6500000 |

Documents

Certificate of Analysis

| | |
|--------------------------|--|
| Enter Lot No. | Anfi |
| Bulk Quote Order Product | S08 |
| Structure Search | Sucrose, Low Endotoxin USP Documentation (188) |

Related Content

Figure 25

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Mannitol, Powder, USP - GenAR®



Formula: $C_6H_{14}O_6$

Formula Weight: 182.17

Suitable for Use in Biotechnology

| Select Size | Product Number | Units per Case | Package Size | Container Type | Price per Unit | Case Price per Unit | Min Order in Units | Quantity (Units) |
|--------------------------|----------------|----------------|--------------|------------------------------------|----------------|---------------------|--------------------|----------------------|
| <input type="checkbox"/> | 7781-88 | 1 | 12KG | 6.5GL polyethylene pail: screw top | \$1467.49 | -- | 1 | <input type="text"/> |

You must be [logged in](#) to add the products to the cart.

* Products noted with an asterisks (*) in the "Units Per Case" column are available for sale in case units of measure only.

The following product(s) have been removed from catalog:

| Removed Products | Package Size | Equivalent Products | Equivalent Pkg Size | Comments/Notes |
|------------------|--------------|---------------------|---------------------|--|
| 7781-04 | 500G | Not Available | -- | Please contact Customer Services . |

Product Specifications

| TEST | SPECIFICATION |
|---|----------------|
| GMP Manufactured Product | |
| Meets B.P. Chemical Specifications | |
| Meets E.P. Chemical Specifications | |
| Meets U.S.P Requirements | |
| CAUTION: For Manufacturing, processing or repackaging | |
| Bulk Pharmaceutical Chemical | |
| USP - Assay (dried basis) | 97.0 - 102.0 % |
| USP - Identification | Passes Test |
| USP - Related Substances: Sorbitol | <= 2.0 % |
| USP - Related Substances: Sum of isomalt& maltitol | <= 2.0 % |
| USP - Related Substances: Unspecified impurities | <= 0.10 % |
| USP - Related Substances: Total impurities | <= 2.0 % |
| USP - Loss on Drying at 105°C | <= 0.5 % |
| USP - Melting Point | 165 - 170 °C |
| USP - Reducing Sugars | <= 0.1 % |
| USP - Nickel (Ni) | <= 1 ppm |
| USP - Appearance of Solution | Passes Test |
| USP - Conductivity (µS cm-1) | <= 20 µS/cm |
| USP - Total Aerobic Microbes | <= 100 cfu/g |

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OK

Figure 25 cont'

| | |
|--|----------------|
| USP - Total Yeast and Mold Count | <= 100 cfu/g |
| USP - Escherichia coli | Passes Test |
| EP/BP - Assay (as C ₁₂ H ₁₄ O ₂) (calculated on anhydrous basis) | 97.0 - 102.0 % |
| EP/BP - Conductivity, uS cm-1 | <= 20 |
| EP/BP - Identification C | Passes Test |
| EP/BP - Melting Point | 165 - 170 °C |
| EP/BP - Heavy Metals (as Pb) | <= 5 ppm |
| EP/BP - Nickel (Ni) | <= 1 ppm |
| EP/BP - Loss on Drying | <= 0.5 % |
| EP/BP - Reducing Sugars | <= 0.1 % |
| EP/BP - Related Substances: Impurity A | <= 2.0 % |
| EP/BP - Related Substances: Sum of Impurities B & C | <= 2.0 % |
| EP/BP - Related Substances: Unspecified Impurities, each | <= 0.10 % |
| EP/BP - Related Substances: Total Impurities | <= 2.0 % |
| EP/BP - Appearance of Solution | Passes Test |
| Total Aerobic Microbial Count (cfu/g) | <= 1000 |
| Total Mold and Yeast Count (cfu/g) | <= 100 |
| EP/BP - Escherichia coli (absent) | Passes Test |
| Salmonella (absent) | Passes Test |
| Endotoxin Concentration (EU/g) | <= 10 |
| Appearance (fine, white crystalline powder) | |
| Not Intended for Parenteral Use | |
| Only Class 2 solvents (Ethylene Glycol) are likely to be present. All are below Option 1 limits. | |
| Metallic Residues: No metal catalysts or metal reagents, as defined by EMA Guideline EMEA/CHMP/SWP/4446/2000, are used in the production of this material. | |

Product Information

| | |
|----------------------|--------------|
| CAS Number | 69-65-8 |
| HTS Number | 2905430000 |
| Density | 1L = 1.52 Kg |
| Management of Change | R |

| | | | | |
|--|--|--|--|--|
| <p>Corporate</p> <p>Distributors</p> <p>Careers</p> <p>Media Contacts & Requests</p> <p>Contact Us</p> <p>Investor Relations</p> | <p>Ordering</p> <p>How to Buy</p> <p>E-Commerce</p> <p>Account Information</p> <p>Terms & Conditions of Sale</p> | <p>Help</p> <p>Contact Us</p> <p>Product Inquiries</p> <p>Ask Avantor® Search FAQ</p> <p>Forgot Password</p> | <p>Product Resources</p> <p>Brands</p> <p>Certificates of Analysis</p> <p>MSDS</p> <p>Product Specifications</p> <p>ISO Certificates</p> | <p>Connect with Us</p> <p>1-800-AVANTOR (1-888-252-6867)</p> <p>Outside U.S.: +1-610-670-2800</p> <p>Other Regions: Contact Us</p> |
|  | | | | |

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SIGMA[®]

Figure 26

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 Fax (314) 286-7828
 email: techserv@sial.com
 sigma-aldrich.com

Product Information

D-(+)-Glucose

 Product Number **G 8270**

Store at Room Temperature

Product Description

 Molecular Formula: C₆H₁₂O₆

CAS Number: 50-99-7

Molecular Weight: 180.2

 Melting Point : 146 °C for α-D-glucose and 150 °C for β-D-glucose¹

 pH: 5.9 (0.5 M aqueous)²

 Densities of aqueous solutions (w/v) at 17.5 °C with respect to water:²

| Concentration (%) | 5 | 10 | 20 | 30 | 40 |
|-------------------|-------|-------|-------|-------|-------|
| Density (g/ml) | 1.019 | 1.038 | 1.076 | 1.113 | 1.149 |

Glucose is a main source of energy for living organisms. Glucose occurs naturally in the free state in fruits and other parts of plants. Glucose is combined into glucosides, disaccharides, oligosaccharides, the polysaccharides (cellulose and starch), and glycogen.

Glucose is a mixture of α- and β-anomers, primarily the α-anomer. The optical rotation of the α-anomer is +112.2° (c = 10% in water, 20 °C) and the β-anomer is +18.7° (c = 10% in water, 20 °C). When D-glucose is dissolved in water, the optical rotation gradually changes (mutarotates) with time and approaches a final equilibrium value of +52.7° (c = 10%, 20 °C) due to the formation of an equilibrium mixture consisting of approximately one-third α- and two-thirds β-D-glucose.²

Normal human blood contains 0.08-0.1% glucose.² Small amounts of glucose (also hydrogen peroxide or glucose oxidase) can be measured using luminol as a substrate with horseradish peroxidase.³

Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

Preparation Instructions

One gram of glucose dissolves in 1.1 ml of water at 25 °C and in 0.18 ml of water at 90 °C.²

Storage/Stability

Solutions are sterilized by autoclaving.⁴

References

1. Biochemistry, 2nd ed., Lehninger, A. L., ed., Worth Publishers, Inc. (New York, NY: 1975), p. 253.
2. The Merck Index, 13th Ed., Entry# 4472.
3. Puget, K., and Michelson, A.M., Microestimation of glucose and glucose oxidase. *Biochimie*, **58**, 757-758 (1976).
4. Martindale The Extra Pharmacopoeia, 29th ed., Reynolds, J. E. F., ed., The Pharmaceutical Press (London, England: 1989), p. 1265.

MES/AJH 11/02

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USA 12022 21025 - Dextran from *Leuconostoc* spp.

SEVA-ALONCH

1238 21025

Dextran from *Leuconostoc* spp.

Figure 27

M_w ~40,000

Synonym: DEXTAN 40

SDS SIMILAR PRODUCTS

CAS Number 9004-64-0 | Linear Formula (C₆H₁₂O₆)_n | EC Number 232-677-6 | MDL number MFCD00130936

Properties

| | |
|--------------------|---|
| Related Categories | Dinucleotides and Fragments, Biotinilation Agents, Carbohydrates, Dextran, Dextran for General Research Applications, Misc. ... |
| mol wt | M _w ~40,000 |
| loss | 27% loss on drying |
| absorption | A 10%/375 ref. H ₂ O 50.05 |
| storage temp | room temp |

Description

Packaging
500 g in poly bottle

Application
Dextran is a branched glucan composed of linear α(1→6) linked glucose units and α(1→3) side-attached branches. Dextran ranges in size from 10,000 to 150,000 kDa. Dextran is used in many applications as volume extenders, stabilizers, matrix components, binding platforms, lubricants and physical structure components.
Dextran 40 is used in the development of new improved preservation solutions for organ transplantation. Dextran 40 may be used to determine cell tightness and flux parameters across cell layers. Dextran 40 is used as a colloid plasma volume extender.

Price and Availability



| SKU-Pack Size | Availability | Price (USD) | Quantity |
|---------------|---|-------------|--------------------------------|
| 31389-100G | Only 6 left in stock (more on the way) - FROM | 296.00 | <input type="text" value="0"/> |
| 31389-25G | Available to ship on 03/30/18 - FROM | 28.50 | <input type="text" value="0"/> |
| 31389-500G | Available to ship on 03/30/18 - FROM | 1,027.00 | <input type="text" value="0"/> |

BULK ORDERS?






ADD TO CART

Figure 27 cont'

Customers Also Viewed

| | | | | |
|--|---|---|--|---|
| <p>68084 Dextran from <i>Leuconostoc</i> spp. M_r ~40000</p>  | <p>1179708 Dextran 40 United States Pharmacopeia (USP) Reference Standard</p> | <p>31388 Dextran from <i>Leuconostoc</i> spp. M_r ~6,000</p> | <p>31390 Dextran from <i>Leuconostoc</i> spp. M_r ~70,000</p>  | <p>09184 Dextran from <i>Leuconostoc</i> spp. M_r ~100,000</p> |
| <p>D1662 Dextran from <i>Leuconostoc mesenteroides</i> average mol wt 35,000-45,000</p> | <p>31392 Dextran from <i>Leuconostoc</i> spp. M_r 450,000-650,000</p> | <p>1179876 Dextran 40 Calibration United States Pharmacopeia (USP) Reference Standard</p> | <p>31387 Dextran from <i>Leuconostoc</i> spp. M_r 15,000-25,000</p> | <p>D2447 Dimethyl Sulfoxide DMSO/Dextran T 40 55% w/v DMSO USP, Ph.Eur. 5% w/v Dextran T 40 USP in NaCl</p> |

Recently Viewed

| | | | | |
|--|--|---|--|--|
|  <p>68270 D-(+)-Glucose ≥99.5% (GC)</p> |  <p>M4125 D-Mannitol ≥98%</p> |  <p>ARK2195B Sucrose, Low Endospore PharmaGrade, USP/NF, Ph Eur. Manufactured under appropriate GMP controls for pharma or biopharmaceutical production.</p> |  <p>L3375 Calcium lactate/monohydrate 98.0-102.0% (EDTA titration)</p> |  <p>153516 Lactobionic acid 97%</p> |
|--|--|---|--|--|

Safety Information

| | |
|-------------------------------|--|
| Personal Protective Equipment | Goggles, Gloves, type N95 (US), type P1 (EN143), respirator filter |
| RIDADR | NONH for all modes of transport |
| WGK Germany | 2 |

Documents

Certificate of Analysis

| | |
|---------------------------|------|
| Enter Lot no. | Anfi |
| Bulk Quote-Order Protocol | 508 |
| Specification Sheet | |

Articles

Dextran and Related Polysaccharides

Historically, dextrans had been long recognized as contaminants in sugar processing and other food production. The formation of dextran in wine was shown by Pasteur to be due to the activity of micro-
Vicki Caligur
BioFiles 2008, 3:10, 17
Keywords: Adhesion, Applications, Chromatography, Clinical, Cosmetic, Derivatizations, Diffusion, Filtration, Immobilization, Industries, Ion Exchange, Ligands, Microscopy, Molecular biology, Molecular biology techniques, Nuclear magnetic resonance spectroscopy, Nucleic acid hybridization, Pharmaceutical, Precipitation, Purification, Separation, Size-exclusion chromatography, Spectroscopy, Substrates.
Type

Peer-Reviewed Papers

Did you use this product in your Paper? If so click here.

Product Information

sigma-aldrich.com

Figure 28

3050 Spruce Street, Saint Louis, MO 63103 USA

Tel: (800) 521-8958 (314) 771-5785 Fax: (314) 771-5052 (314) 771-5787

email: techserv@sigmaaldrich.com sigma-aldrich.com

Adenosine

Product Number **A9251**

Storage Temperature 2-8 °C

Product Description

Molecular Formula: $C_{10}H_{13}N_5O_4$

Molecular Weight: 267.2

CAS Number: 58-61-7

Specific Rotation: -61.7° (7 mg/100 ml water)¹ pK_a : 3.5 and 12.5.²Extinction Coefficient: $E^{1\%1cm} = 15.4$ (259 nm in 0.01 M PO_4 , pH 7)³

Adenosine is a nucleoside, a purine riboside widely distributed in nature. Adenosine is the product of the degradation of AMP.

Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

Preparation Instructions

Soluble in water (7 mg/ml).¹

Storage/Stability

Adenosine readily hydrolyzes in dilute mineral acids to adenine and D-ribose. Nitrous acid causes deamination.²

References

1. The Merck Index, 11th ed., Entry# 143
2. Dawson, R.M. C., et al., Data for Biochemical Research, 3rd ed., p. 77., Oxford University Press, New York, (1986).
3. Specifications and Criteria for Biochemical Compounds, 3rd ed., p. 157.

MES/ 8/08

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SIGMA-ALDRICH® Figure 29

sigma-aldrich.com

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Website: www.sigmaaldrich.comEmail USA: techserv@sial.comOutside USA: eurtechserv@sial.com

Product Specification

Product Name:

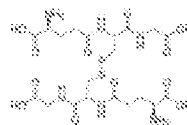
L-Glutathione oxidized - PharmaGrade, Manufactured under appropriate controls for use as a raw material in pharma or biopharmaceutical production.

Product Number:

G2299

CAS Number:

27025-41-8


Formula:
C₂₀H₃₂N₆O₁₂S₂
Formula Weight:

612.63 g/mol

Storage Temperature:

2 - 8 °C

TEST
Specification

| | |
|-----------------------------|-----------------------|
| Appearance (Color) | White |
| Appearance (Form) | Powder |
| Solubility (Color) | Colorless |
| Solubility (Turbidity) | Clear |
| 10 mg/ml, H ₂ O | |
| Infrared spectrum | Conforms to Structure |
| Water (by Karl Fischer) | ≤ 6 % |
| Nitrogen (anhydrous) | 13.4 - 14.0 % |
| Purity (HPLC) | ≥ 98 % |
| Impurity | ≤ 2 % |
| Glutathione reduced by HPLC | |
| Recommended Retest Period | ----- |
| 3 Years | |

Specification: PRD.0.ZQ5.10000041806

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

HypoThermosol® FRS**Figure 30**Scientists Helping Scientists™ | WWW.STEMCELL.COM

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Animal Component-Free, Defined Hypothermic (2 - 8°C) Preservation Medium

| | | |
|-----------|-------|---------------|
| Catalog # | 07935 | 100 mL |
| | 07934 | 16 x 10 mL |
| | 07936 | 500 mL Bottle |
| | 07945 | 500 mL Bag |

Product Description

HypoThermosol® FRS is an optimized hypothermic (2 - 8°C) preservation media that enables improved and extended preservation of cells, tissues and organs. HypoThermosol® FRS is uniquely formulated to address the molecular-biological response of cells during the hypothermic preservation process. HypoThermosol® FRS includes key ions at concentrations that balance the intracellular state at hypothermic temperatures. Additional components include pH buffers, energy substrates, free radical scavengers, and osmotic/oncotic stabilizers.

- Ready-to-use
- Serum-free, protein-free
- Animal component-free
- cGMP manufactured with USP grade / highest quality components
- FDA master file
- Sterility, endotoxin, and cell-based release testing

Properties

Storage: Store at 2 - 8°C.

Shelf Life: Stable until expiry date (EXP) on label. Product should be protected from prolonged exposure to light.

Please refer to the Safety Data Sheet (SDS) for hazard information.

Product may be shipped at room temperature (15 - 25°C) and should be refrigerated upon receipt.

Handling / Directions For Use

HypoThermosol® FRS is ready to use and contains protective agents.

1. Wipe down outside of HypoThermosol® FRS container with 70% ethanol or isopropanol before opening.
2. Completely replace culture medium with cold (2 - 8°C) HypoThermosol® FRS. Do not warm HypoThermosol® FRS to 37°C.
3. Maintain sample at 2 - 8°C for duration of cold storage. HypoThermosol® FRS is not a culture medium, do not use above 15°C.

At the end of cold storage, simply centrifuge the cells to pellet them and remove HypoThermosol® FRS medium. Replace HypoThermosol® FRS with warmed culture medium of choice and flick the tube gently to resuspend the cells.

THIS PRODUCT IS MANUFACTURED UNDER A cGMP QUALITY MANAGEMENT SYSTEM COMPLIANT TO 21 CFR 820.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2019/050197

A. CLASSIFICATION OF SUBJECT MATTER**A01N 1/02 (2006.01) A61K 35/28 (2015.01) A61K 35/51 (2015.01) A61P 17/02 (2006.01)**

According to International Patent Classification (IPC)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A01N, A61K, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPODOC/WPI/TXTE/REGISTRY/CAPLUS/EMBASE/BIOSIS/MEDLINE: Trolox, Hypothermosol, HTS-FRS, hypothermic, transport, shipping, preservation, stem cells, mesenchymal stem cells, MSCs, UC-MSCs, CLSCs, corlicyte and related terms; CAS REGISTRY NUMBER: 53188-07-1

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| X | WO 2010/064054 A1 (RENEURON LIMITED) 10 June 2010 Whole document, particularly page 5, lines 6-7, 25-26, page 6, lines 3-4, 12-18, page 8, lines 7-24, page 10, lines 5-21, page 11, lines 9-11 | 1-50 |
| X | WILLIAMS L.B. ET AL., Response to intravenous allogeneic equine cord Blood-Derived Mesenchymal stromal cells administered from chilled or Frozen state in serum and Protein-Free Media. <i>Front. Vet. Sci.</i> , 22 July 2016, Vol. 3, No. 56, pages 1-13 [Retrieved on 2019-06-12] <DOI: 10.3389/FVETS.2016.00056> Whole document, particularly Materials and Methods; Fig. 1B; page 2, right col. para. 1, page 3, right col. para. 1, page 3, left col. last para., page 10, left col. para. 1 | 1-50 |
| X | WO 2015/175457 A1 (ROWLEY, J.A. AND LOCK L.T.) 19 November 2015 Whole document, particularly page 6, lines 10-11, 23-26, page 15, lines 2-6, 10-15, page 23, lines 23-28, page 25, lines 18-19; Example 4; Fig. 15 | 1-50 |

 Further documents are listed in the continuation of Box C. See patent family annex.***Special categories of cited documents:**

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

12/06/2019

(day/month/year)

Date of mailing of the international search report

15/07/2019

(day/month/year)

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Authorized officer

Chen Xiuli (Dr)

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2019/050197

| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|---|--|-----------------------|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | GINIS I. ET AL., Evaluation of Bone Marrow-Derived Mesenchymal Stem Cells After Cryopreservation and Hypothermic Storage in Clinically Safe Medium. <i>TISSUE ENGINEERING: Part C</i> , 30 January 2012, Vol. 18, No. 6, pages 453-463 [Retrieved on 2019-06-12] <DOI: 10.1089/TEN.TEC.2011.0395> Whole document, particularly page 453, right col. para. 2, page 462, left col., Materials and Methods; Fig. 4; Table 5 | 1-50 |
| X | WO 2007/046775 A1 (CELL-RESEARCH CORPORATION PTE LTD) 26 April 2007 Whole document, in particular para. [0015], [00234], [00241]; Examples 2, 23-26; Fig. 32-37 | 33-36, 38, 41-50 |
| X | WO 2006/019357 A1 (CELL-RESEARCH CORPORATION PTE LTD) 23 February 2006 Whole document, in particular para. [0052], [0055], [0058] [0084]; Claims 13, 14, 16 and 21; Examples 2 & 4 | 33-36, 38, 41-50 |
| X | WO 2017/096607 A1 (LI C. & GUO L.) 15 June 2017 Whole document, particularly page 15; Figure 3 of the machine translation | 33-36, 38, 41-50 |
| A | BAUST J.M., Chapter 5: Advances in Media for Cryopreservation and Hypothermic Storage. <i>BioProcess International</i> , 1 June 2005, No. Supplement , pages 46-56 [Retrieved on 2019-06-12] <DOI: NOT AVAILABLE> Whole document, in particular page 46, left column para. 1, page 48, right col. para. 2-3, page 49, left col. to center col. para. 1, page 50, right col. para. 1; Fig. 2 | - |
| P,X | HARRISON R. ET AL., Development and validation of broadspectrum magnetic particle labelling processes for cell therapy manufacturing. <i>Stem Cell Research & Therapy</i> , 26 September 2018, Vol. 9, No. 248, pages 1-13 [Retrieved on 2019-06-12] <DOI: 10.1186/S13287-018-0968-0> Abstract, page 4, left col., last para., page 5, right col.). | 1-50 |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2019/050197

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. forming part of the international application as filed:
 - in the form of an Annex C/ST.25 text file.
 - on paper or in the form of an image file.
 - b. furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. furnished subsequent to the international filing date for the purposes of international search only:
 - in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
 - on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Although a sequence listing has been filed or furnished, it was not used for the purposes of this search.

Since only one version or copy of a sequence listing has been filed or furnished, the statements under item 2 are not required.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2019/050197

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:

because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please refer to Supplemental Box (Continuation of Box No. III).

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

No protest accompanied the payment of additional search fees.

Supplemental Box
(Continuation of Box No. III)

This International Searching Authority found multiple inventions in this international application, as follows:

Group 1: Claims 1-32, 37, 39 and 40 relate to a liquid carrier comprising specific components (i)-(viii) as used in a method of transporting a stem cell population (claims 1-32, 39 and 40) or which contained a unit dose of mesenchymal stem cell population (claim 37).

Group 2: Claims 33-36, 38 and 41-50 relate to a mesenchymal stem cell population, specifically in a unit dosage or in a therapeutic use thereof.

Please refer to **Box No. IV** of Written Opinion of The International Searching Authority (Form PCT/ISA/237) for detailed explanation.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/SG2019/050197

Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.

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| WO 2017/096607 A1 | 15/06/2017 | NONE | |