- Activin A, and - $VEGF_{165}$; wherein the cell culture medium of step b is for initiating differentiation of pluripotent stem cells into hematopoietic precursor cells, thereby generating hematopoietic precursor cells from pluripotent stem cells using microcarrier embryoid bodies (EB) or pluripotent stem cells, the cell culture media comprising – BMP4, - Activin A, and - a vascular endothelial growth factor; wherein the cell culture medium of step c is for differentiation of pluripotent stem cells into hematopoietic precursor cells, thereby generating hematopoietic precursor cells from pluripotent stem cells using microcarrier embryoid bodies (EB) or pluripotent stem cells, the cell culture media comprising - BMP4, - Activin A, - bFGF (basic fibroblast growth factor), - beta-estradiol, - stem cell factor (SCF), and - a vascular endothelial growth factor.

[0010] In another example, the present disclosure refers to a method for differentiation of pluripotent stem cells into hematopoietic precursor cells, wherein the method is carried out under suspension agitation, the method comprising optionally providing pluripotent stem cells; inducing mesoderm induction in the pluripotent stem cells isolated from step a. according to the method as disclosed herein, thereby resulting in KDR+PDGFRα- hematopoietic precursor cells; inducing hematopoietic induction in the cells isolated from step b, thereby resulting in CD34/CD43/CD45 hematopoietic progenitor cells; inducing erythroblast expansion in the cells isolated from step c, thereby resulting in CD235a+CD71+ erythroblast cells; inducing erythroblast maturation in the cells isolated from step d, thereby resulting in CD235a+ DRAQ5-ve enucleated erythroblast cells; removing the cell culture medium, and isolating the resulting CD235a+ DRAQ5-ve enucleated erythroblast cells of step e.

[0011] In a further example, the present disclosure refers to a kit comprising micro-carriers and the cell culture media as disclosed herein.

CLAIMS

1. A method of differentiation of pluripotent stem cells into hematopoietic precursor cells, wherein the method is carried out under continuous suspension agitation, wherein the method comprises use of a cell culture media during the mesoderm induction stage, the cell culture media comprising

- BMP4,
- CHIR99021,
- Activin A, and
- $VEGF_{165}$.

2. The method of claim 1, wherein BMP4 is present in a concentration of between 26 to 36 ng/ml, wherein Activin A is present in a concentration of between 35 to 46 ng/ml, wherein CHIR-99021 is present in a concentration of between 8 µM to 14 µM, and wherein $VEGF_{165}$ is present in a concentration of between 48 ng/ml to 51 ng/ml.

3. A method for differentiation of pluripotent stem cells into hematopoietic precursor cells, wherein the method is carried out under continuous suspension agitation, the method comprising

a. exposing pluripotent stem cells to a cell culture medium for 24 hours (day 0 to day 1), thereby resulting in T-Brachyury (T-Bra; primitive streak/early mesoderm marker) positive cells;

b. exposing the cells of step a. to a further cell culture medium for 24 hours (day 1 to day 2);

c. exposing the micro-carrier attached cells of step b. to another further cell culture medium for 48 hours (day 2 to day 4), whereby steps a. to b. induce mesoderm induction;

d. removing the cell culture medium, and isolating the resulting $KDR^{+}PDGFR\alpha^{-}$ hematopoietic precursor cells of step c;

wherein the cell culture medium of step a is for initiating differentiation of pluripotent stem cells into hematopoietic precursor cells, thereby generating hematopoietic precursor cells from pluripotent stem cells using microcarrier embryoid bodies (EB), the cell culture media comprising

- BMP4,
- CHIR99021,
- Activin A, and
- $VEGF_{165}$;

wherein the cell culture medium of step b is for initiating differentiation of pluripotent stem cells into hematopoietic precursor cells, thereby generating hematopoietic precursor cells from pluripotent stem cells using microcarrier embryoid bodies (EB) or pluripotent stem cells, the cell culture media comprising

- BMP4,
- Activin A, and
- a vascular endothelial growth factor;

wherein the cell culture medium of step c is for differentiation of pluripotent stem cells into hematopoietic precursor cells, thereby generating hematopoietic precursor cells from pluripotent stem cells using microcarrier embryoid bodies (EB) or pluripotent stem cells, the cell culture media comprising

- BMP4,
- Activin A,
- bFGF (basic fibroblast growth factor),
- beta-estradiol,
- stem cell factor (SCF), and
- a vascular endothelial growth factor.

4. The method of claim 3, wherein for the cell culture medium of step a: BMP4 is present in a concentration of between 26 ng/ml to 36 ng/ml, wherein Activin A is present in a concentration of between 35 to 46 ng/ml, wherein CHIR99021 is present in a concentration of between 8 µM to 14 µM, and wherein $VEGF_{165}$ is present in a concentration of between 48 ng/ml to 51 ng/ml.

5. The method of claim 3, wherein for the cell culture medium of step b: the vascular endothelial growth factor of the cell culture media of step b is $VEGF_{165}$.

6. The method of claim 5, wherein BMP4 is present in a concentration of between 26 to 36 ng/ml, wherein Activin A is present in a concentration of between 35 to 46 ng/ml, and wherein $VEGF_{165}$ is present in a concentration of between 48 ng/ml to 51 ng/ml.

7. The method of claim 3, wherein for the cell culture medium of step c: wherein the basic fibroblast growth factor (bFGF) is a heat-stable chimeric variant of bFGF or a stable chimeric fibroblast growth factor (FGF); and/or wherein the vascular endothelial growth factor is $VEGF_{165}$.

8. The method of claim 7, wherein BMP4 is present in a concentration of between 18 ng/ml to 27 ng/ml, wherein Activin A is present in a concentration of between 3 ng/ml to 7 ng/ml, wherein the bFGF is present in a concentration of between 5 ng/ml to 14 g/ml, wherein the beta-estradiol is present in a concentration of between 0.2 ng/ml to 0.8 ng/ml, wherein the SCF is present in a concentration of between 18 ng/ml to 27 ng/ml, and wherein $VEGF_{165}$ is present in a concentration of between 20 ng/ml to 35 ng/ml.