defined, and scalable methods for generating human arterial endothelial cells from human pluripotent stem cells.

SUMMARY

[0006] Disclosed herein is a method of obtaining arterial endothelial cells. The method can comprise or consist essentially of culturing mesodermal cells in a serum-free, albumin-free, chemically defined culture medium that is substantially free of insulin and comprises a fibroblast growth factor (FGF), a vascular endothelial growth factor (VEGF), and at least one of a Notch agonist, a TGF-beta inhibitor, and an inhibitor of inositol monophosphatase, whereby a cell population comprising arterial endothelial cells is obtained. The arterial endothelial cells of the population can express one or more markers selected from the group consisting of Ephrin2, neuropilin1 (NRP-1), Delta-like 4 (DLL4), ephrin-B2 (EFNB2), CD44, CXCR4/CD184, Gap Junction Protein Alpha-4 (GJA4), Hey1, Jagged-1 (JAG1), Notch1, and Notch4. The cell population can comprise at least 80% arterial endothelial cells. In a first aspect, the present disclosure refers to a method of obtaining human arterial endothelial cells, the method comprising culturing human mesodermal cells in a serum-free, albumin-free, chemically defined culture medium that is substantially free of insulin and comprises a fibroblast growth factor (FGF), a vascular endothelial growth factor (VEGF), and at least one of a Notch agonist, a TGF-beta inhibitor, and an inhibitor of inositol monophosphatase, whereby a cell population comprising at least 80% human arterial endothelial cells is obtained, wherein arterial endothelial cells of the population express activated Ephrin B2 (EFNB2), KLF3, neuropilin1 (NRP-1), Delta-like 4 (DLL4), CXCR4/CD184, Gap Junction Protein Alpha-4 (GJA4), Gap Junction Protein Alpha-5 (GJA5), and Hey1, and one or more markers selected from the group consisting of Jagged-1 (JAG1), Notch1, and Notch4, wherein CXCR4, DLL4, EFNB2, GJA4, GJA5, and HEY1 are expressed at a level at least 10X greater than that of human umbilical vein endothelial cells (HUVECs).

[0007] The serum-free, albumin-free, chemically defined culture medium can comprise a FGF, a VEGF, a Notch agonist, a TGF-beta inhibitor, and an inhibitor of inositol monophosphatase. The mesodermal cells can express one or more mesodermal markers selected from the group consisting of *Brachyury (T)*, *EMOS*, *FOXA2*, *MIXL1*, *MSX1*, and *MSX2*.

[0008] In some cases, the mesodermal cells are obtained by culturing human pluripotent stem cells for a period of about two days in a serum-free, albumin-free, chemically defined cell culture medium comprising a Bone Morphogenetic Protein (BMP), Activin A, and an activator of Wnt/β-catenin signaling to obtain a cell population comprising mesodermal cells. The mesodermal cells can express one or more mesodermal markers selected from the group consisting of *Brachyury (T)*, *EMOS*, *FOXA2*, *MIXL1*, *MSX1*, and *MSX2*. The pluripotent stem cells can be human embryonic stem cells or human induced pluripotent stem cells. The activator of Wnt/β-catenin signaling can be a Gsk3 inhibitor. The Gsk3 inhibitor can be selected from the group consisting of CHIR 99021, CHIR 98014, BIO-acetoxime, BIO, LiCl, SB 216763, SB 415286, AR A014418, 1-Azakenpaullone, and Bis-7-indolylmaleimide. The Notch agonist can be selected from the group consisting of Resveratrol (3,4',5-trihydroxystilbene), valproic acid, and suberoyl bishydroxamic acid. The TGF-beta inhibitor can be SB431542. The inhibitor of inositol monophosphatase can be L-690,330.

[0009] In another aspect, the present disclosure refers to a substantially pure, isolated population of arterial endothelial cells obtained according to a method provided herein, wherein the arterial endothelial cells are in a serum-free, albumin-free, chemically defined culture medium. The isolated population can comprise at least 90% arterial endothelial cells or at least 99% arterial endothelial cells.

[00010] In another aspect, the present disclosure refers to a substantially pure, isolated population of pluripotent stem cell-derived arterial endothelial cells obtained according to a method provided herein, wherein the arterial endothelial cells are in a serum-free, albumin-free, chemically defined culture medium. The isolated population can comprise at least 90% arterial endothelial cells or at least 99% arterial endothelial cells.

[00011] Disclosed is a method of in vitro screening of agents. The method can comprise contacting a test agent to arterial endothelial cells obtained according to a method provided herein; and detecting an effect of the agent on the contacted arterial endothelial cells. In a further aspect, the present disclosure refers to a method of *in vitro* screening of an agent, comprising (a) contacting a test agent to arterial endothelial cells obtained according to a method provided herein, wherein the arterial endothelial cells are in a serum-free, albumin-free, chemically defined culture medium; and (b) detecting an effect of the agent on adhesion of leukocytes to the contacted arterial endothelial cells. Detecting can comprise performing a method selected from the group consisting of RNA sequencing, gene expression profiling, transcriptome analysis, metabolome analysis, detecting reporter or sensor, protein expression profiling, Förster resonance energy transfer (FRET), metabolic profiling, and microdialysis. In another aspect, the present disclosure refers to a method for vascularizing an engineered tissue construct, the method comprising contacting arterial endothelial cells obtained according to a method provided herein to an engineered tissue construct.

[00012] In yet another aspect, the present disclosure refers to a kit when used in a method of obtaining human arterial endothelial cells, the kit comprising: (i) a serum-free, albumin-free, chemically defined culture medium suitable for differentiation of mesodermal cells into arterial endothelial cells, wherein the culture medium is substantially free of insulin and comprises a fibroblast growth factor (FGF), a vascular endothelial growth factor (VEGF), and at least one of a Notch agonist, a TGF-beta inhibitor, and an inhibitor of inositol monophosphatase; and (ii) instructions describing a method for differentiating mesodermal cells into arterial endothelial cells, the method employing the culture medium, wherein the method comprises culturing human mesodermal cells in the serum-free, albumin-free, chemically defined culture medium that is substantially free of insulin and comprises a fibroblast growth factor (FGF), a vascular endothelial growth factor (VEGF), and at least one of a Notch agonist, a TGF-beta inhibitor, and an inhibitor of inositol monophosphatase, whereby a cell population comprising at least 80% human arterial endothelial cells is obtained, wherein arterial endothelial cells of the population express activated Ephrin B2 (EFNB2), KLF3, neuropilin1 (NRP-1), Delta-like 4 (DLL4), CXCR4/CD184, Gap Junction Protein Alpha-4 (GJA4), Gap Junction Protein Alpha-5 (GJA5), and Hey1, and one or more markers selected from the group consisting of Jagged-1 (JAG1), Notch1, and Notch4, and wherein CXCR4, DLL4, EFNB2, GJA4, GJA5, and HEY1 are expressed at a level at least 10X greater than that of human umbilical vein endothelial cells (HUVECs). The kit can further comprise (a) a serum-free, albumin-free, chemically defined culture medium suitable for differentiation of human pluripotent stem cells into mesodermal cells, where the culture medium comprises a BMP, Activin A, and an activator of Wnt/β-catenin signaling; and (b) instructions describing a method for differentiating human pluripotent stem cells into arterial endothelial cells, the method employing the culture medium of (a).

[00013] These and other features, aspects, and advantages of the present disclosure will become better understood from the description that follows. In the description, reference is made to the accompanying drawings, which form a part hereof and in which there is shown by way of illustration, not limitation, embodiments of the disclosure. The description of preferred embodiments is not intended to limit the disclosure to cover all modifications, equivalents and

CLAIMS

We claim:

1. A method of obtaining human arterial endothelial cells, the method comprising

culturing human mesodermal cells in a serum-free, albumin-free, chemically defined culture medium that is substantially free of insulin and comprises a fibroblast growth factor (FGF), a vascular endothelial growth factor (VEGF), and at least one of a Notch agonist, a TGF-beta inhibitor, and an inhibitor of inositol monophosphatase, whereby a cell population comprising at least 80% human arterial endothelial cells is obtained,

wherein arterial endothelial cells of the population express activated Ephrin B2 (EFNB2), KLF3, neuropilin1 (NRP-1), Delta-like 4 (DLL4), CXCR4/CD184, Gap Junction Protein Alpha-4 (GJA4), Gap Junction Protein Alpha-5 (GJA5), and Hey1, and one or more markers selected from the group consisting of Jagged-1 (JAG1), Notch1, and Notch4,

wherein CXCR4, DLL4, EFNB2, GJA4, GJA5, and HEY1 are expressed at a level at least 10X greater than that of human umbilical vein endothelial cells (HUVECs).

2. The method of claim 1, wherein CXCR4, DLL4, GJA4, and HEY1 are expressed at a level at least 50X greater than that of HUVECs.

3. The method of claim 1, wherein the serum-free, albumin-free, chemically defined culture medium comprises a FGF, a VEGF, a Notch agonist, a TGF-beta inhibitor, and an inhibitor of inositol monophosphatase.

4. The method of claim 1, wherein the mesodermal cells are selected from the group consisting of: (a) mesodermal cells expressing one or more mesodermal markers selected from the group consisting of *Brachyury (T)*, *EMOS*, *FOXA2*, *MIXL1*, *MSX1*, and *MSX2*; and

(b) mesodermal cells that are obtained by culturing human pluripotent stem cells for a period of about two days in a serum-free, albumin-free, chemically defined cell culture medium comprising a Bone Morphogenetic Protein (BMP), Activin A, and an activator of Wnt/β-catenin signaling to obtain a cell population comprising mesodermal cells.

5. The method of claim 4 part (b), wherein the mesodermal cells express one or more mesodermal markers selected from the group consisting of *Brachyury (T)*, *EMOS*, *FOXA2*, *MIXL1*, *MSX1*, and *MSX2*.

6. The method of claim 4 part (b), wherein the pluripotent stem cells are human embryonic stem cells or human induced pluripotent stem cells.

7. The method of claim 4 part (b), wherein the activator of Wnt/β-catenin signaling is a Gsk3 inhibitor.

8. The method of claim 7, wherein the Gsk3 inhibitor is selected from the group consisting of CHIR 99021, CHIR 98014, BIO-acetoxime, BIO, LiCl, SB 216763, SB 415286, AR A014418, 1-Azakenpaullone, and Bis-7-indolylmaleimide.

9. The method of claim 4 part (b), wherein the Notch agonist is selected from the group consisting of Resveratrol (3,4',5-trihydroxystilbene), valproic acid, and suberoyl bishydroxamic acid.

10. The method of claim 4 part (b), wherein the TGF-beta inhibitor is selected from the group consisting of SB431542, SB-525334, A83-01, LY2157299, LY210976, GW788388, RepSox, SB-505124, D4476, SD208, and EW-7197.

11. The method of claim 4 part (b), wherein the inhibitor of inositol monophosphatase is selected from the group consisting of L-690,330, PI3K inhibitor LY294002, Pictilisib, HS-173, GSK2636771, Duvelisib, TG100-115, GSK1059615, PF-04691502, PIK-93, BGT226, AZD6482, SAR245409, BYL719, CUDC-907, IC-87114, TG100713, Gedatolisib, CH5132799, PKI-402, BAY 80-6946, XL147, PIK-90, PIK-293, Quercetin, Wortmannin, ZSTK474. AS-252424, AS-604850, and Apitolisib.

12. A substantially pure, isolated population of arterial endothelial cells obtained according to the method of claim 1, wherein the arterial endothelial cells are in a serum-free, albumin-free, chemically defined culture medium.

13. A substantially pure, isolated population of pluripotent stem cell-derived arterial endothelial cells obtained according to the method of claim 4 part (b), wherein the arterial endothelial cells are in a serum-free, albumin-free, chemically defined culture medium.

14. The isolated population of claim 12 or 13, selected from the group consisting of (i) an isolated population comprising at least 90% arterial endothelial cells, and

(ii) an isolated population comprising at least 99% arterial endothelial cells.

15. A method of *in vitro* screening of an agent, comprising

(a) contacting a test agent to arterial endothelial cells obtained according to the method of claim 1, wherein the arterial endothelial cells are in a serum-free, albumin-free, chemically defined culture medium; and

(b) detecting an effect of the agent on adhesion of leukocytes to the contacted arterial endothelial cells.

16. The method of claim 15, wherein detecting comprises performing a method selected from the group consisting of a leukocyte adhesion assay, RNA sequencing, gene expression profiling, transcriptome analysis, metabolome analysis, detecting reporter or sensor, protein expression profiling, Förster resonance energy transfer (FRET), metabolic profiling, and microdialysis.

17. A method for vascularizing an engineered tissue construct, the method comprising contacting arterial endothelial cells obtained according to the method of claim 1 to an engineered tissue construct.

18. The method of claim 17, wherein the engineered tissue construct comprises vascular smooth muscle cells.

19. A kit when used in a method of obtaining human arterial endothelial cells, the kit comprising: (i) a serum-free, albumin-free, chemically defined culture medium suitable for differentiation of mesodermal cells into arterial endothelial cells, wherein the culture medium is substantially free of insulin and comprises a fibroblast growth factor (FGF), a vascular endothelial growth factor (VEGF), and at least one of a Notch agonist, a TGF-beta inhibitor, and an inhibitor of inositol monophosphatase; and (ii) instructions describing a method for differentiating mesodermal cells into arterial endothelial cells, the method employing the culture medium,

wherein the method comprises culturing human mesodermal cells in the serum-free, albumin-free, chemically defined culture medium that is substantially free of insulin and comprises a fibroblast growth factor (FGF), a vascular endothelial growth factor (VEGF), and at least one of a Notch agonist, a TGF-beta inhibitor, and an inhibitor of inositol monophosphatase, whereby a cell population comprising at least 80% human arterial endothelial cells is obtained,

wherein arterial endothelial cells of the population express activated Ephrin B2 (EFNB2), KLF3, neuropilin1 (NRP-1), Delta-like 4 (DLL4), CXCR4/CD184, Gap Junction Protein Alpha-4 (GJA4), Gap Junction Protein Alpha-5 (GJA5), and Hey1, and one or more markers selected from the group consisting of Jagged-1 (JAG1), Notch1, and Notch4, and

wherein CXCR4, DLL4, EFNB2, GJA4, GJA5, and HEY1 are expressed at a level at least 10X greater than that of human umbilical vein endothelial cells (HUVECs).

20. The kit of claim 19, further comprising:

(a) a serum-free, albumin-free, chemically defined culture medium suitable for differentiation of human pluripotent stem cells into mesodermal cells, wherein the culture medium comprises a BMP, Activin A, and an activator of Wnt/β-catenin signaling; and

(b) instructions describing a method for differentiating human pluripotent stem cells into mesodermal cells, the method comprising culturing human pluripotent stem cells for a period of about two days in a serum-free, albumin-free, chemically defined cell culture medium comprising a Bone Morphogenetic Protein (BMP), Activin A, and an activator of Wnt/β-catenin signaling whereby a cell population comprising mesodermal cells is obtained.