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, provided herein is a method of obtaining arterial endothelial cells, the method comprising 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, wherein arterial endothelial cells of the population express one or more markers selected from the group consisting of Ephrin B2 (EFNB2), neuropilin1 (NRP-1), Delta-like 4 (DLL4), CD44, CXCR4/CD184, Gap Junction Protein Alpha-4 (GJA4), Hey1, Jagged-1 (JAG1), Notch1, and Notch4.

[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, provided herein is a substantially pure, isolated population of arterial endothelial cells obtained according to a method provided herein. The isolated population can comprise at least 90% arterial endothelial cells or at least 99% arterial endothelial cells.

[00010] In another aspect, provided herein is a substantially pure, isolated population of pluripotent stem cell-derived arterial endothelial cells obtained according to a method provided herein. 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, provided herein is 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; 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, provided herein is 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, provided herein is a kit for obtaining 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. 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

alternatives. Reference should therefore be made to the claims recited herein for interpreting the scope of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[00014] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the compositions and methods provided herein. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[00015] FIGS. 1A-1D demonstrate single-cell RNA-seq. (A) Hierarchical clustering analysis of arterial and venous gene of single cells. (B) Average arterial and venous gene expression of five cell subpopulations. In each subpopulation, the average TPM (transcript per million) of each gene was calculated and normalized to population P1 (arterial genes) or P3 (venous genes). The normalized expression of all arterial gene or venous genes was further averaged and shown in a bar graph. Data were represented as mean ±SD. *: $P<0.05$, $n=13$ cells in P1, $n=10$ cells in P3. (C) Principal component analysis of P1 and P3. The plot was generated by Singular™ Analysis Toolset 2.1. (D) Arterial enriched genes. The mean TPM of each gene for P1 was compared to P3 to calculate fold change. *P*-value was also calculated by comparing P1 to P3. Arterial enriched genes were identified with fold change>2, *P* value<0.1, and the mean TPM of P1 >10. $P<0.1$ was used as the cut off since the *P* value of the previously reported arterial genes, VEGFa, Fzd4, Fzd7, Fzd10, Dll4, and Notch4, was between 0.01 to 0.1.

[00016] FIG. 2A provides a schematic representation of a protocol for generating arterial endothelial cells from human pluripotent stem cells using chemically-defined media listed in Table 1. Human embryonic stem (ES) cells were first differentiated into mesoderm cells using E8BAC medium. E6 medium supplemented with growth factors or small molecules (E6FVB) was then used to induce mesoderm cells to differentiate into endothelial cells. FIG. 2B is flow cytometric data for of CD31 and CD34 expression at day 0 (undifferentiated pluripotent state) and at day 5 (differentiated state). FIG. 2C is flow cytometric data for KDR, NANOG, and OCT4 expression at day 0 and at day 5. FIG. 2D indicates CD144 expression on purified endothelial cells by immunostaining. FIG. 2E shows a LDL (low density lipoprotein) uptake assay. FIG. 2F shows an *in vitro* MATRIGEL® (BD Biosciences, Falcon®) encapsulation assay. FIG. 2G shows the results of an *in vivo* MATRIGEL® gel plug assay for analysis of migration

CLAIMS

We claim:

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

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,

wherein arterial endothelial cells of the population express one or more markers selected from the group consisting of Ephrin B2 (EFNB2), neuropilin1 (NRP-1), Delta-like 4 (DLL4), CD44, CXCR4/CD184, Gap Junction Protein Alpha-4 (GJA4), Hey1, Jagged-1 (JAG1), Notch1, and Notch4.

2. The method of claim 1, wherein the cell population comprises at least 80% arterial endothelial cells.

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 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 SB431542.

11. The method of claim 4 part (b), wherein the inhibitor of inositol monophosphatase is L-690,330.

12. A substantially pure, isolated population of arterial endothelial cells obtained according to the method of claim 1.

13. A substantially pure, isolated population of pluripotent stem cell-derived arterial endothelial cells obtained according to the method of claim 4 part (b).

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; 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 for obtaining 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.

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 arterial endothelial cells, the method employing the culture medium of (a).