SUMMARY

[0008] The present disclosure provides a methods of treating a vascular disease comprising administering to a subject a composition comprising hemogenic endothelial cells (HEs) obtained by *in vitro* differentiation of pluripotent stem cells.

[0008a] In one aspect, the present disclosure provides the use of a composition in the manufacture of a medicament for treating a vascular disease in a subject suffering from, or suspected of suffering from, a vascular disease, wherein the composition comprises hemogenic endothelial cells (HEs) obtained by in vitro differentiation of pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; and wherein the HE cells are CD73(+).

[0008b] In another aspect, the present disclosure provides a composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; wherein the HEs are obtained by *in vitro* differentiation of pluripotent stem cells in the absence of TGFβ inhibitors; and wherein the HEs are CD73(+), CD43(-), CD45(-), and CD146 (+).

[0008c] In another aspect, the present disclosure provides a composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; wherein the HEs are obtained by *in vitro* differentiation of pluripotent stem cells in the absence of TGFβ inhibitors; and wherein the HEs express CD144 (VECAD) and CD73.

[0008d] In another aspect, the present disclosure provides a pharmaceutical composition comprising the HEs as disclosed herein.

[0008e] In another aspect, the present disclosure provides a pharmaceutical composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells and a pharmaceutically acceptable carrier, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; wherein the HEs are obtained by *in vitro* differentiation of pluripotent stem cells in the absence of TGFβ inhibitors; and wherein the HEs express CD73, CD144 (VECAD), CD31, and CD309/KDR (FLK-1).

[0009] In an example, the vascular disease is selected from the group consisting of coronary artery diseases (e.g., arteriosclerosis, atherosclerosis, and other diseases or injuries of the arteries, arterioles and capillaries or related complaint), myocardial infarction, (e.g. acute myocardial infarction), organizing myocardial infarct, ischemic heart disease, arrhythmia, left ventricular dilatation, emboli, heart failure, congestive heart failure, subendocardial fibrosis, left or right ventricular hypertrophy, myocarditis, chronic coronary

ischemia, dilated cardiomyopathy, restenosis, arrhythmia, angina, hypertension (e.g. pulmonary hypertension, glomerular hypertension, portal hypertension), myocardial hypertrophy, peripheral arterial disease including critical limb ischemia, cerebrovascular disease, renal artery stenosis, aortic aneurysm, pulmonary heart disease, cardiac dysrhythmias, inflammatory heart disease, congenital heart disease, rheumatic heart disease, diabetic vascular diseases, and endothelial lung injury diseases (e.g., acute lung injury (ALI), acute respiratory distress syndrome (ARDS)). In a specific example, the vascular disease is pulmonary hypertension. In another example, the vascular disease is pulmonary arterial hypertension.

[0010] In an example of any of the methods disclosed herein, the mean pulmonary (artery) pressure is reduced in the subject.

[0011] The present disclosure also provides a method of increasing blood flow in pulmonary arteries comprising administering to a subject a composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells. In an example, the subject has pulmonary hypertension. In a specific example, the subject has pulmonary arterial hypertension.

[0012] The present disclosure further provides a method of reducing blood pressure in a subject comprising administering to the subject a composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells. In an example, the subject has pulmonary hypertension. In a specific example, the subject has pulmonary arterial hypertension. In another example, the blood pressure is diastolic pressure. In yet another example, the blood pressure is systolic pressure. In a further example, the blood pressure is mean pulmonary (artery) pressure. Moreover, the blood pressure may be reduced by at least 20% in the subject by any of the methods of the present disclosure.

[0013] In an example, the pluripotent stem cells disclosed herein are embryonic stem cells. In another example, the pluripotent stem cells disclosed herein are induced pluripotent stem cells.

[0014] In yet another example, the HEs disclosed herein are obtained by culturing the pluripotent stem cells under adherent conditions in a differentiation medium in the absence of methylcellulose. In another example, the HEs disclosed herein are obtained by *in vitro* differentiation of pluripotent stem cells without embryoid body formation.

[0015] In any of the methods provided herein, the subject may be a human. Additionally, the pluripotent stem cells disclosed herein may be human pluripotent stem cells. Furthermore, the HEs disclosed herein may be human HEs.

[0016] The HEs disclosed herein may be positive for at least one microRNA (miRNA) selected from the group consisting of miRNA-126, mi-RNA-24, miRNA-196-b, miRNA-214, miRNA-199a-3p, miRNA-335, hsa-miR-11399, hsa-miR-196b-3p, hsa-miR-5690, and hsa-miR-7151-3p. In an example, the HEs are positive for (i) miRNA-214, miRNA-199a-3p, and miRNA-335 and/or (ii) hsa-miR-11399, hsa-miR-196b-3p, hsa-miR-5690, and hsa-miR-7151-3p. In another example, the HEs are positive for (i) miRNA-126, miRNA-24, miRNA-196-b, miRNA-214, miRNA-199a-3p, and miRNA-335 and/or (ii) hsa-miR-11399, hsa-miR-196b-3p, hsa-miR-5690, and hsa-miR-7151-3p. In an example, the HEs are positive for miRNA-214.

[0017] In any of the examples, the HEs disclosed herein may be negative for at least one miRNA selected from the group consisting of miRNA-367, miRNA-302a, miRNA-302b, miRNA-302c, miRNA-223, and miRNA-142-3p. In an example, the HEs are negative for miRNA-223, and miRNA-142-3p. In another example, the HEs are negative for miRNA-367, miRNA-302a, miRNA-302b, miRNA-302c, miRNA-223, and miRNA-142-3p.

[0018] In an example, the HEs are positive for miRNA-214, miRNA-199a-3p, and miRNA-335, and negative for miRNA-223, and miRNA-142-3p.

[0019] In an example, any of the HEs disclosed herein express at least one cell surface marker selected from the group consisting of CD31/PECAM1, CD309/KDR, CD144, CD34, CXCR4, CD146, Tie2, CD140b, CD90, CD271, and CD105. In an example, the HEs of the disclosure express CD146, CXCR4, CD309/KDR, CD90, and CD271. In another example, the HEs of the disclosure express CD146. In an example, the HEs of the disclosure express CD31/PECAM1, CD309/KDR, CD144, CD34, and CD105.

[0020] In an example, the HEs exhibit limited or no detection of at least one cell surface marker selected from the group consisting of CD34, CXCR7, CD43 and CD45. In another

example, the HEs exhibit limited or no detection of CXCR7, CD43, and CD45. In another example, the HEs exhibit limited or no detection of CD43 and CD45.

[0021] In an example, the HEs of the disclosure are CD43(-), CD45(-), and/or CD146 (+). In another example, HEs express CD31, Calponin (CNN1), and NG2, and therefore have the potential to differentiate into endothelial (CD31+), smooth muscle (Calponin+) and /or pericyte (NG2+) cells.

[0022] In an example, CD144 (VECAD)-expressing HEs are isolated from the HEs of the disclosures. In an example, the isolated CD144 (VECAD)-expressing HE cells further express CD31 and/or CD309/KDR (FLK-1). In another example, the isolated CD144 (VECAD)-expressing HE cells further express at least one gene listed in Table 22 and Table 23. In another example, the isolated CD144 (VECAD)-expressing HE cells further express at least one cell marker selected from the group consisting of PLVAP, GJA4, ESAM, EGFL7, KDR/VEGFR2, and ESAM. In an example, the isolated CD144 (VECAD)-expressing HE cells further express at least one cell marker selected from the group consisting of SOX9, PDGFRA, and EGFRA. In another example, the isolated CD144 (VECAD)-expressing HE cells further express at least one cell marker selected from the group consisting of KDR/VEGFR2, NOTCH4, collagen I, and collagen IV. In an example, the composition comprising CD144 (VECAD)-expressing HEs isolated from the HEs of the disclosure substantially lack CD144 (VECAD)-negative HEs.

[0023] Accordingly, the present disclosure also provides a composition comprising HEs obtained by *in vitr*o differentiation of pluripotent stem cells disclosed herein. The present disclosure further provides a pharmaceutical composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells disclosed herein and a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] **FIG. 1** is an overview of an exemplary method for producing HEs.

[0025] **FIG. 2** is an overview of an exemplary method for producing hemangioblasts (HBs).

[0026] **FIG. 3** are bar graphs of PDGFRa, HAND1, FOXF1, APLNR, PECAM/CD31 expression in cells over the course the differentiation process from ES cells (day -1). Time points tested were at day -1 (ES cells), day 2 (D2), day 4 (D4), and day 6 (D6).

[0027] **FIG. 4A** shows a graph of CD31, CD43, CD34, KDR, CXCR4, CD144, CD146, and CD105 expression in J1-HE cells (red, left bar) and GMP1-HE cells (blue, right bar) obtained at day 6 of the differentiation process.

CLAIMS

What is claimed is:

1. Use of a composition in the manufacture of a medicament for treating a vascular disease in a subject suffering from, or suspected of suffering from, a vascular disease, wherein the composition comprises hemogenic endothelial cells (HEs) obtained by *in vitro* differentiation of pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; and wherein the HE cells are CD73(+).

2. The use of claim 1, wherein the vascular disease is selected from the group consisting of coronary artery diseases (e.g., arteriosclerosis, atherosclerosis, and other diseases or injuries of the arteries, arterioles and capillaries or related complaint), myocardial infarction, (e.g. acute myocardial infarction), organizing myocardial infarct, ischemic heart disease, arrhythmia, left ventricular dilatation, emboli, heart failure, congestive heart failure, subendocardial fibrosis, left or right ventricular hypertrophy, myocarditis, chronic coronary ischemia, dilated cardiomyopathy, restenosis, arrhythmia, angina, hypertension (eg. pulmonary hypertension, glomerular hypertension, portal hypertension), myocardial hypertrophy, peripheral arterial disease including critical limb ischemia, cerebrovascular disease, renal artery stenosis, aortic aneurysm, pulmonary heart disease, cardiac dysrhythmias, inflammatory heart disease, congenital heart disease, rheumatic heart disease, diabetic vascular diseases, endothelial lung injury diseases (e.g., acute lung injury (ALI), and acute respiratory distress syndrome (ARDS)).

3. The use of claim 1, wherein the vascular disease is pulmonary hypertension or pulmonary arterial hypertension.

4. The use of any one of claims 1-3, wherein the mean pulmonary (artery) pressure is reduced in the subject.

5. Use of a composition in the manufacture of a medicament for increasing blood flow in pulmonary arteries in a subject suffering from, or suspected of suffering from, a vascular disease, wherein the composition comprises HEs obtained by *in vitro* differentiation of

pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; and wherein the HE cells are CD73(+).

6. The use of claim 5, wherein the subject has pulmonary hypertension or pulmonary arterial hypertension.

7. Use of a composition in the manufacture of a medicament for reducing blood pressure in a subject suffering from, or suspected of suffering from, a vascular disease, wherein the composition comprises HEs obtained by *in vitro* differentiation of pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; and wherein the HE cells are CD73(+).

8. The use of claim 7, wherein the subject has pulmonary hypertension or pulmonary arterial hypertension.

9. The use of claim 7, wherein the blood pressure is diastolic pressure, systolic pressure, and/or mean pulmonary (artery) pressure.

10. The use of any one of claims 7-9, wherein the blood pressure is reduced by at least 20% in the subject.

11. The use of any one of claims 1-10, wherein

(a) the HEs are positive for at least one microRNA (miRNA) selected from the group consisting of miRNA-126, miRNA-24, miRNA-196-b, miRNA-214, miRNA-199a-3p, miRNA-335, hsa-miR-11399, hsa-miR-196b-3p, hsa-miR-5690, and hsa-miR-7151-3p;

(b) the HEs are positive for (i) miRNA-214, miRNA-199a-3p, and miRNA-335 and/or (ii) hsa-miR-11399, hsa-miR-196b-3p, hsa-miR-5690, and hsa-miR-7151-3p;

(c) the HEs are positive for (i) miRNA-126, miRNA-24, miRNA-196-b, miRNA-214, miRNA-199a-3p, and miRNA-335 and/or (ii) hsa-miR-11399, hsa-miR-196b-3p, hsa-miR-5690, and hsa-miR-7151-3p;

(d) the HEs are positive for miRNA-214;

(e) the HEs are negative for at least one miRNA selected from the group consisting of miRNA-367, miRNA-302a, miRNA-302b, miRNA-302c, miRNA-223, and miRNA-142-3p;

(f) the HEs are negative for miRNA-223, and miRNA-142-3p;

(g) the HEs are negative for miRNA-367, miRNA-302a, miRNA-302b, miRNA-302c, miRNA-223, and miRNA-142-3p;

(h) the HEs further express at least one cell surface marker selected from the group consisting of CD24, CD31/PECAM1, CD309/KDR, CD144, CD34, CXCR4, CD146, Tie2, CD140b, CD90, CD271, and CD105;

(i) the HEs express CD146, CXCR4, CD309/KDR, CD90, and CD271;

(j)the HEs express CD146;

(k) the HEs express CD144 (VECAD);

(l) the HEs further express at least one cell marker selected from the group consisting of CD24, CD31, CD309/KDR (FLK-1), PLVAP, GJA4, EGFL7, KDR/VEGFR2, and ESAM;

(m) the HEs further express at least one cell marker selected from the group consisting of SOX9, PDGFRA, and EGFRA;

(n) the HEs further express at least one cell marker selected from the group consisting of KDR/VEGFR2, NOTCH4, collagen I, and collagen IV;

(o) the HEs express CD31/PECAM1, CD309/KDR, CD144, CD34, and CD105;

(p) the HEs exhibit limited or no detection of at least one cell surface marker selected from the group consisting of CD34, CXCR7, CD43 and CD45;

(q) the HEs exhibit limited or no detection of CXCR7, CD43, and CD45;

(r) the HEs exhibit limited or no detection of CD43 and CD45;

(s) the HEs are CD73(+), CD43(-), CD45(-), and CD146 (+);

(t) the HEs express (i) CD144 (VECAD) and (ii) CD73 and (iii) CD31 and/or CD309/KDR (FLK-1);

(u) the HEs express at least one gene listed in Table 22 and Table 23; and/or

(v) the HE further express CD24.

12. The use of any one of claims 1-11, wherein the HEs are obtained (i) by culturing the pluripotent stem cells under adherent conditions in a differentiation medium in the absence of methylcellulose; and/or (ii) by in vitro differentiation of pluripotent stem cells without embryoid body formation.

13. The use of any one of claims 1-12, wherein the subject is a human.

14. A composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; wherein the HEs are obtained by *in vitro* differentiation of pluripotent stem cells in the absence of TGFβ inhibitors; and wherein the HEs are CD73(+), CD43(-), CD45(-), and CD146 (+).

15. The composition of claim 14, wherein

(a) the HEs are positive for at least one microRNA (miRNA) selected from the group consisting of miRNA-126, mi-RNA-24, miRNA-196-b, miRNA-214, miRNA-199a-3p, miRNA-335, hsa-miR-11399, hsa-miR-196b-3p, hsa-miR-5690, and hsa-miR-7151-3p;

(b) the HEs are positive for (i) miRNA-214, miRNA-199a-3p, and miRNA-335 and/or (ii) hsa-miR-11399, hsa-miR-196b-3p, hsa-miR-5690, and hsa-miR-7151-3p;

(c) the HEs are positive for (i) miRNA-126, mi-RNA-24, miRNA-196-b, miRNA-214, miRNA-199a-3p, and miRNA-335 and/or (ii) hsa-miR-11399, hsa-miR-196b-3p, hsa-miR-5690, and hsa-miR-7151-3p;

(d) the HEs are positive for miRNA-214;

(e) the HEs are negative for at least one miRNA selected from the group consisting of miRNA-367, miRNA-302a, miRNA-302b, miRNA-302c, miRNA-223, and miRNA-142-3p;

(f) the HEs are negative for miRNA-223, and miRNA-142-3p; and/or

(g) the HEs are negative for miRNA-367, miRNA-302a, miRNA-302b, miRNA-302c, miRNA-223, and miRNA-142-3p.

16. A composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; wherein the HEs are obtained by *in vitro* differentiation of pluripotent stem cells in the absence of TGFβ inhibitors; and wherein the HEs express CD144 (VECAD) and CD73.

17. The composition of claim 16, wherein

(a) the HEs further express at least one cell marker selected from the group consisting of CD24, CD31, CD309/KDR (FLK-1), PLVAP, GJA4, EGFL7, KDR/VEGFR2, and ESAM;

(b) the HEs further express at least one cell marker selected from the group consisting of SOX9, PDGFRA, and EGFRA;

(c) the HEs further express at least one cell marker selected from the group consisting of KDR/VEGFR2, NOTCH4, collagen I, and collagen IV; and/or

(d) the composition substantially lacks CD73-negative and CD144 (VECAD)-negative HE cells; and/or

(e) the HEs express (i) CD144 (VECAD) and (ii) CD73 and (iii) CD31 and/or CD309/KDR (FLK-1);

(f) the HEs express at least one gene listed in Table 22 and Table 23; and/or

(g) the HE further express CD24.

18. A pharmaceutical composition comprising the HEs of any one of claims 14-17.

19. A pharmaceutical composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells and a pharmaceutically acceptable carrier, wherein the pluripotent stem cells comprise embryonic stem cells and/or induced pluripotent stem cells; wherein the HEs are obtained by *in vitro* differentiation of pluripotent stem cells in the absence of TGFβ inhibitors; and wherein the HEs express CD73, CD144 (VECAD), CD31, and CD309/KDR (FLK-1).

20. The pharmaceutical composition of claim 19, wherein

(a) the HEs further express at least one cell marker selected from the group consisting of CD24, CD31, CD309/KDR (FLK-1), PLVAP, GJA4, EGFL7, KDR/VEGFR2, and ESAM;

(b) the HEs further express at least one cell marker selected from the group consisting of SOX9, PDGFRA, and EGFRA;

(c) the HEs further express at least one cell marker selected from the group consisting of KDR/VEGFR2, NOTCH4, collagen I, and collagen IV;

(d) the composition substantially lacks CD73-negative and CD144 (VECAD)-negative HE cells;

(e) the HEs express (i) CD144 (VECAD) and (ii) CD73 and (iii) CD31 and/or CD309/KDR (FLK-1);

(f) the HEs further express at least one gene listed in Table 22 and Table 23; and/or

(g) the HEs further express CD24.

21. The use of any one of claims 1-12, wherein the HEs are obtained by *in vitro* differentiation of pluripotent stem cells in the absence of TGFβ inhibitors.