

CLAIMS

What is claimed is:

1. A method of treating a vascular disease in a subject suffering from, or suspected of suffering from, a vascular disease, comprising administering to the subject a composition comprising hemogenic endothelial cells (HEs) obtained by *in vitro* differentiation of pluripotent stem cells.
2. The method 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 method of claim 1, wherein the vascular disease is pulmonary hypertension.
4. The method of claim 1, wherein the vascular disease is pulmonary arterial hypertension.
5. The method of any one of claims 1-4, wherein the mean pulmonary (artery) pressure is reduced in the subject.
6. A method of increasing blood flow in pulmonary arteries in a subject suffering from, or suspected of suffering from, a vascular disease, comprising administering to the subject a composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells.
7. The method of claim 6, wherein the subject has pulmonary hypertension.
8. The method of claim 6, wherein the subject has pulmonary arterial hypertension.

9. A method of reducing blood pressure in a subject suffering from, or suspected of suffering from, a vascular disease, comprising administering to the subject a composition comprising HEs obtained by in vitro differentiation of pluripotent stem cells.
10. The method of claim 9, wherein the subject has pulmonary hypertension.
11. The method of claim 9, wherein the subject has pulmonary arterial hypertension.
12. The method of claim 9, wherein the blood pressure is diastolic pressure.
13. The method of claim 9, wherein the blood pressure is systolic pressure.
14. The method of claim 9, wherein the blood pressure is mean pulmonary (artery) pressure.
15. The method of any one of claims 9-14, wherein the blood pressure is reduced by at least 20% in the subject.
16. The method of any one of claims 1-15, wherein 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.
17. The method of claim 16, wherein 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.
18. The method of claim 16, wherein 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.
19. The method of claim 16, wherein the HEs are positive for miRNA-214.
20. The method of any one of claims 1-19, wherein 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.
21. The method of claim 20, wherein the HEs are negative for miRNA-223, and miRNA-142-3p.

22. The method of claim 20, wherein the HEs are negative for miRNA-367, miRNA-302a, miRNA-302b, miRNA-302c, miRNA-223, and miRNA-142-3p.
23. The method of any one of claims 1-22, wherein the HEs 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.
24. The method of claim 23, wherein the HEs express CD146, CXCR4, CD309/KDR, CD90, and CD271.
25. The method of claim 23, wherein the HEs express CD146.
26. The method of claim 23, wherein the HEs express CD144 (VECAD).
27. The method of claim 26, wherein HEs express at least one cell marker selected from the group consisting of CD31, CD309/KDR (FLK-1), PLVAP, GJA4, ESAM, EGFL7, KDR/VEGFR2, and ESAM.
28. The method of claim 26 or 27, wherein the HEs further express at least one cell marker selected from the group consisting of SOX9, PDGFRA, and EGFR.
29. The method of any one of claims 26-28, wherein the HEs further express at least one cell marker selected from the group consisting of KDR/VEGFR2, NOTCH4, collagen I, and collagen IV.
30. The method of claim 23, wherein the HEs express CD31/PECAM1, CD309/KDR, CD144, CD34, and CD105.
31. The method of any one of claims 1-29, wherein 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.
32. The method of any one of claims 1-31, wherein the HEs exhibit limited or no detection of CXCR7, CD43, and CD45.
33. The method of any one of claims 1-31, wherein the HEs exhibit limited or no detection of CD43 and CD45.

34. The method of any one of claims 1-33, wherein the HEs are CD43(-), CD45(-), and CD146 (+).
35. The method of any one of claims 1-34, wherein the pluripotent stem cells is embryonic stem cells.
36. The method of any one of claims 1-34, wherein the pluripotent stem cells is induced pluripotent stem cells.
37. The method of any one of claims 1-36, wherein the HEs are obtained by culturing the pluripotent stem cells under adherent conditions in a differentiation medium in the absence of methylcellulose.
38. The method of any one of claims 1-37, wherein the HEs are obtained by *in vitro* differentiation of pluripotent stem cells without embryoid body formation.
39. The method of any one of claims 1-38, wherein the subject is a human.
40. The method of any one of claims 1-39, wherein the pluripotent stem cells are human pluripotent stem cells.
41. The method of any one of claims 1-40, wherein the HEs are human HEs.
42. A composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells, wherein the HEs are CD43(-), CD45(-), and CD146 (+).
43. A composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells, wherein 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.
44. The composition of claim 43, wherein 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.
45. The composition of claim 43, wherein 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.

46. The composition of claim 43, wherein the HEs are positive for miRNA-214.
47. The composition of any one of claims 42-46, wherein 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.
48. The composition of claim 47, wherein the HEs are negative for miRNA-223, and miRNA-142-3p.
49. The composition of claim 47, wherein the HEs are negative for miRNA-367, miRNA-302a, miRNA-302b, miRNA-302c, miRNA-223, and miRNA-142-3p.
50. The composition of any one of claims 43-49, wherein the HEs are CD43(-), CD45(-), and CD146 (+).
51. A composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells, wherein the HEs express CD144 (VECAD).
52. The composition of claim 51, wherein HEs further express at least one cell marker selected from the group consisting of CD31, CD309/KDR (FLK-1), PLVAP, GJA4, ESAM, EGFL7, KDR/VEGFR2, and ESAM.
53. The composition of claim 51 or 52, wherein the HEs further express at least one cell marker selected from the group consisting of SOX9, PDGFRA, and EGFR.
54. The composition of any one of claims 51-53, wherein the HEs further express at least one cell marker selected from the group consisting of KDR/VEGFR2, NOTCH4, collagen I, and collagen IV.
55. The composition of any one of claims 51-54, wherein the composition substantially lacks CD144 (VECAD)-negative HE cells.
56. A pharmaceutical composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells and a pharmaceutically acceptable carrier, wherein the HEs are CD43(-), CD45(-), and CD146 (+).
57. A pharmaceutical composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells and a pharmaceutically acceptable carrier, wherein 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.

58. The pharmaceutical composition of claim 57, wherein 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.

59. The pharmaceutical composition of claim 57, wherein 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.

60. The pharmaceutical composition of claim 57, wherein the HEs are positive for miRNA-214.

61. The pharmaceutical composition of any one of claims 57-60, wherein 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.

62. The pharmaceutical composition of claim 61, wherein the HEs are negative for miRNA-223, and miRNA-142-3p.

63. The pharmaceutical composition of claim 61, wherein the HEs are negative for miRNA-367, miRNA-302a, miRNA-302b, miRNA-302c, miRNA-223, and miRNA-142-3p.

64. The pharmaceutical composition of any one of claims 57-63, wherein the HEs are CD43(-), CD45(-), and CD146 (+).

65. A pharmaceutical composition comprising HEs obtained by *in vitro* differentiation of pluripotent stem cells and a pharmaceutically acceptable carrier, wherein the HEs express CD144 (VECAD), CD31, and CD309/KDR (FLK-1).

66. The pharmaceutical composition of claim 65, wherein HEs further express at least one cell marker selected from the group consisting of CD31, CD309/KDR (FLK-1), PLVAP, GJA4, ESAM, EGFL7, KDR/VEGFR2, and ESAM.

67. The pharmaceutical composition of claim 65 or 66, wherein the HEs further express at least one cell marker selected from the group consisting of SOX9, PDGFRA, and EGFR.

68. The pharmaceutical composition of any one of claims 65-67, wherein the HEs further express at least one cell marker selected from the group consisting of KDR/VEGFR2, NOTCH4, collagen I, and collagen IV.
69. The pharmaceutical composition of any one of claims 65-68, wherein the composition substantially lacks CD144 (VECAD)-negative HE cells.
70. The method of any one of claims 26-41, wherein the HEs express (i) CD144 (VECAD) and (ii) CD31 and/or CD309/KDR (FLK-1).
71. The method of any one of claims 26-41 or 70, wherein the HEs express at least one gene listed in Table 22 and Table 23.
72. The composition of any one of claims 51-54, wherein the HEs express (i) CD144 (VECAD) and (ii) CD31 and/or CD309/KDR (FLK-1).
73. The composition of any one of claims 51-54 or 72, wherein the HEs express at least one gene listed in Table 22 and Table 23.
74. The pharmaceutical composition of any one of claims 65-69, wherein the HEs express (i) CD144 (VECAD) and (ii) CD31 and/or CD309/KDR (FLK-1).
75. The pharmaceutical composition of any one of claims 65-69 or 74, wherein the HEs express at least one gene listed in Table 22 and Table 23.