Summary

[007] The present disclosure relates to novel methods of producing mesoderm-derived vascular progenitor cells (meso-VPCs) by *in vitro* differentiation of pluripotent stem cells. The present disclosure further provides methods of treating vascular diseases, *e.g.*, critical limb ischemia, using the meso-VPCs of the current disclosure.

[007a] In one aspect, provided herein is a method of producing a population of mesoderm-derived vascular progenitor cells (meso-VPCs) from pluripotent stem cells, wherein the method comprises

(a) culturing the pluripotent stem cells on an extracellular matrix surface, in a mesoderm differentiation medium comprising Activin-A, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and bone morphogenetic protein 4 (BMP4), for 1 day,

(b) culturing the cells of step (a) on an extracellular matrix surface, in a second mesoderm differentiation medium comprising VEGF, FGF and BMP4, for 3 to 5 days, to produce mesoderm cells, and

(c) culturing the mesoderm cells derived from the pluripotent stem cells under non-adherent or low adherent conditions, in a meso-VPC differentiation medium comprising VEGF, FGF, BMP4, and a small molecule inhibitor of transforming growth factor-beta (TGF-β) type I receptor, for 3 days to 7 days, thereby producing a population of mesoderm-derived vascular progenitor cells (meso-VPCs).

[007b] In another aspect, provided herein is a method producing a population of mesoderm-derived vascular progenitor cell (meso-VPC) from pluripotent stem cells, wherein the method comprises

(a) culturing the pluripotent stem cells on an extracellular matrix surface, in a first mesoderm differentiation medium comprising Activin-A, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and bone morphogenetic protein 4 (BMP4), for 1 day,

(b) culturing the cells of step (a) in a second mesoderm differentiation medium comprising VEGF, FGF and BMP4, for 3 to 5 days, to produce mesoderm cells,

(c) culturing the mesoderm cells derived from the pluripotent stem cells from step (b) on an extracellular matrix surface, in a third medium comprising VEGF, FGF, and BMP4, for 1 day, and

(d) culturing the cells produced in step (c) on an extracellular matrix surface, in a fourth medium comprising VEGF, FGF, BMP4, and a small molecule inhibitor of transforming growth factor-beta (TGF-β) type I receptor, for 4 days to 7 days,

thereby producing the population of mesoderm-derived vascular progenitor cells.

[007c] In another aspect, provided herein is a composition comprising comprising a population of mesoderm-derived vascular progenitor cells (meso-VPCs) produced by the method as described herein, wherein the population of meso-VPCs expresses at least one cell-surface marker selected from

the group consisting of CD31/PECAM1, CD309/KDR, CD43, CD144, CD34, CD184/CXCR4, CD146, and PDGFRb.

[007d] In another aspect, provided herein is a composition comprising a population of meso-VPC produced by the method as described herein, wherein the meso-VPC is positive for at least one miRNA marker selected from the group consisting of mir126, mir125a-5p, mir24, and mir483-5p.

[008] Accordingly, in one aspect, the present disclosure provides a method of producing a population of mesoderm-derived vascular progenitor cells (meso-VPCs) from a pluripotent stem cell, wherein the method comprises culturing a mesoderm cell derived from a pluripotent stem cell under non-adherent or low adherent conditions, in a medium comprising one or more factors selected from the group consisting of vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), bone morphogenetic protein 4 (BMP4), and a small molecule inhibitor of transforming growth factor-beta (TGF-β) type I receptor, thereby producing a population of mesoderm-derived vascular progenitor cells (meso-VPCs).

[009] In one example, the mesoderm cell is derived from a pluripotent stem cell by culturing the pluripotent stem cell in a medium comprising one or more mesoderm inducing growth factors selected from the group consisting of Activin-A, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and bone morphogenetic protein 4 (BMP4).

[010] In one example, the meso-VPCS are produced as a vasculonoid. In another example, the meso-VPCs are dissociated into single cells.

[011] In one example, the mesoderm inducing growth factors comprise Activin-A, VEGF165, FGF-2 and BMP4. In one example, the Activin-A is used at a concentration of about 5-15 ng/mL. In one example, the VEGF165 is used at a concentration of about 5-25 ng/mL. In one example, the FGF-2 is used at a concentration of about 5-25 ng/mL. In one example, the BMP4 is used at a concentration of about 5-50 ng/mL. In one example, the method further comprises removing Activin-A from the culture media after about 24 hours of culturing.

[012] In one example, the pluripotent stem cells are cultured on an extracellular matrix surface. In one example, the extracellular matrix surface is a Matrigel-coated surface. In one example, the pluripotent stem cells are cultured for about 3 days to about 5 days.

[013] In one example, the small molecule inhibitor of transforming growth factor-beta (TGF-β) type I receptor is SB431542. In another example, the one or more factors comprise VEGF165, FGF-2, BMP4, and SB431542. In one example, the one or more factors further comprises Forskolin. In one example, the Forskolin is used at a

Claims

1. A method of producing a population of mesoderm-derived vascular progenitor cells (meso-VPCs) from pluripotent stem cells, wherein the method comprises

(a) culturing the pluripotent stem cells on an extracellular matrix surface, in a mesoderm differentiation medium comprising Activin-A, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and bone morphogenetic protein 4 (BMP4), for 1 day,

(b) culturing the cells of step (a) on an extracellular matrix surface, in a second mesoderm differentiation medium comprising VEGF, FGF and BMP4, for 3 to 5 days, to produce mesoderm cells, and

(c) culturing the mesoderm cells derived from the pluripotent stem cells under non-adherent or low adherent conditions, in a meso-VPC differentiation medium comprising VEGF, FGF, BMP4, and a small molecule inhibitor of transforming growth factor-beta (TGF-β) type I receptor, for 3 days to 7 days, thereby producing a population of mesoderm-derived vascular progenitor cells (meso-VPCs).

2. The method of claim 1, wherein the first mesoderm differentiation medium comprises 5-15 ng/mL Activin-A, 5-25 ng/mL VEGF, 5-25 ng/mL FGF, and 5-50 ng/mL BMP4.

3. The method of claim 1, wherein the meso-VPCS are produced as a vasculonoid, optionally, wherein the method further comprises dissociating the meso-VPCs in the vasculonoid into single cells.

4. The method of claim 1 or 2, wherein the first mesoderm differentiation medium comprises 10 ng/mL Activin-A, 10 ng/mL VEGF165, 10 ng/mL FGF-2, and 25 ng/mL BMP4.

5. The method of claim 1, wherein the second mesoderm differentiation medium comprises 5-25 ng/mL VEGF, 5-25 ng/mL FGF, and 5-50 ng/mL BMP4.

6. The method of claim 1 or 5, wherein the second mesoderm differentiation medium comprises 10 ng/mL VEGF165, 10 ng/mL FGF-2, and 25 ng/mL BMP4.

7. The method of claim 1,

(a) wherein the extracellular matrix surface in steps (a) and (b) is a Matrigel-coated surface; and/or

(b) wherein the non-adherent or low adherent conditions are on an ultra-low attachment surface.

8. The method of claim 1,

(i) wherein the meso-VPC differentiation medium comprises 10-50 ng/mL VEGF, 10-50 ng/mL FGF, 10-50 ng/mL BMP4, and 5-20 μM of a small molecule inhibitor of transforming growth factor-beta (TGF-β) type I receptor;

(ii) wherein the small molecule inhibitor of transforming growth factor-beta (TGF-β) type I receptor is SB431542;

(iii) wherein the meso-VPC differentiation medium comprises 50 ng/mL VEGF165, 50 ng/mL FGF-2, 25 ng/mL BMP4, and 10 μM SB431542;

(iv) wherein the meso-VPC differentiation medium further comprises Forskolin, and/or

(v) wherein the Forskolin is used at a concentration of 2-10 μM.

9. The method of claim 1,

(a) wherein the mesoderm cells in step (c) are cultured under a normoxia condition of 5% CO_2 and 20% O_2; and/or

(b) wherein the pluripotent stem cells in steps (a) and (b) are cultured under a normoxia condition of 5% CO_2 and 20% O_2.

10. A method of producing a population of mesoderm-derived vascular progenitor cell (meso-VPC) from pluripotent stem cells, wherein the method comprises
(a) culturing the pluripotent stem cells on an extracellular matrix surface, in a first mesoderm differentiation medium comprising Activin-A, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and bone morphogenetic protein 4 (BMP4), for 1 day,

(b) culturing the cells of step (a) in a second mesoderm differentiation medium comprising VEGF, FGF and BMP4, for 3 to 5 days, to produce mesoderm cells,
(c) culturing the mesoderm cells derived from the pluripotent stem cells from step (b) on an extracellular matrix surface, in a third medium comprising VEGF, FGF, and BMP4, for 1 day, and
(d) culturing the cells produced in step (c) on an extracellular matrix surface, in a fourth medium comprising VEGF, FGF, BMP4, and a small molecule inhibitor of transforming growth factor-beta (TGF-β) type I receptor, for 4 days to 7 days, thereby producing the population of mesoderm-derived vascular progenitor cells.

11. The method of claim 10, wherein the first mesoderm differentiation medium comprises 5-15 ng/mL Activin-A, 5-25 ng/mL VEGF, 5-25 ng/mL FGF and 5-50 ng/mL BMP4.

12. The method of claim 10, wherein the method further comprises dissociating the population of meso-VPCs into single cells.

13. The method of claim 10 or 11, wherein the first mesoderm differentiation medium comprises 10 ng/mL Activin-A, 10 ng/mL VEGF165, 10 ng/mL FGF-2, and 25 ng/mL BMP4.

14. The method of claim 10, wherein the second mesoderm differentiation medium comprises 5-25 ng/mL VEGF, 5-25 ng/mL FGF, and 5-50 ng/mL BMP4.

15. The method of claim 10 or 14, wherein the second mesoderm differentiation medium comprises 10 ng/mL VEGF165, 10 ng/mL FGF-2, and 25 ng/mL BMP4.

16. The method of claim 10,

(a) wherein the extracellular matrix surface in steps (a) and (b) is a Matrigel®-coated surface;

(b) wherein the extracellular matrix surface in steps (c) and (d) is a collagen-IV-coated surface;

c) wherein the cells in steps (a), (b) and (c) are cultured under a normoxia condition of 5% CO_2 and 20% O_2, and/or

(d) wherein the cells in step (d) are cultured under a hypoxia condition of 5% CO_2 and 5% O_2.

17. The method of claim 10,

(i) wherein the third medium comprises 10-50 ng/mL VEGF, 10-50 ng/mL FGF, and 10-50 ng/mL BMP4;

(ii) wherein the third medium comprise comprises 50 ng/mL VEGF165, 50 ng/mL FGF-2, and 25 ng/mL BMP4;

(iii) wherein the fourth medium comprises 10-50 ng/mL VEGF, 10-50 ng/mL FGF, 10-50 ng/mL BMP4, and 5-20 μM of a small molecule inhibitor of transforming growth factor-beta (TGF-β) type I receptor;

(iv) wherein the small molecule inhibitor of transforming growth factor-beta (TGF-β) type I receptor is SB431542;

(v) wherein the fourth medium comprises 50 ng/mL VEGF165, 50 ng/mL FGF-2, 25 ng/mL BMP4, and 10 μM SB431542;

(vi) wherein the third medium and/or the fourth medium further comprise Forskolin, and/or

(vii) wherein the Forskolin is used at a concentration of 2-10 μM.

18. The method of any one of claims 1-17, wherein the pluripotent stem cells are human embryonic stem cells or human induced pluripotent stem cells.

19. The method of any one of claims 1-18, wherein the population of meso-VPCs

(i) expresses at least one of the cell-surface markers selected from the group consisting of CD31/PECAM1, CD309/KDR, CD43, CD144, CD34, CD184/CXCR4, CD146, and PDGFRb;

(ii) expresses cell-surface markers CD146, CD31/PECAM1, and CD309/KDR;.

(iii) expresses CD31/PECAM1, CD309/KDR, CD146, and (a) at least one of CD144, CD34, CD184/CXCR4, CD43, or PDGFRb, (b) CD34, CD184/CXCR4, and PDGFRb, (c)

CD184/CXCR4, (d) PDGFRb, (e) CD144 and CD184/CXCR4, (f) CD184/CXCR4 and CD43, or (g) CC184/CXCFR4;

(iv) exhibits limited or no detection of (a) one or more of cell-surface markers selected from the group consisting of CXCR7, CD45, and NG2; or (b) CXCR7, CD45, and NG2;

(v) expresses at least one miRNA marker selected from hsa-miR-3917, hsa-miR-450a-2-3p, hsa-miR-542-5p, hsa-miR-126-5p, hsa-miR-125a-5p, hsa-miR-24-3p, hsa-let-7e-5p, hsa-miR-99a-5p, hsa-miR-223-5p, hsa-miR-142-3p, hsa-miR-483-5p, hsa-miR-483-3p, miR 214, miR 335-3p, and miR-199a-3p;

(vi) exhibits limited or no expression of at least one miRNA marker selected from hsa-let-7e-3p, hsa-miR-99a-3p, hsa-miR-133a-5p, hsa-miR-11399, hsa-miR-196b-3p, hsa-miR-5690, and hsa-miR-7151-3p;

(vii) expresses hsa-miR-3917, hsa-miR-450a-2-3p, and hsa-miR-542-5p;

(viii) comprises at least one meso-VPC positive for at least one miRNA markers selected from the group consisting of mir126, mir125a-5p, mir24, and mir483-5p; and/or

(ix) comprises at least one meso-VPC that exhibits limited or no expression for at least one miRNA markers selected from the group consisting of mir367, mir302a, mir302b, mir302c, mirLet7-e, mir223, mir99a, mir142-3p, and mir133a.

20. The method of any one of claims 1-19, further comprising producing a vascular endothelial cell by differentiation of the meso-VPC, optionally, wherein the differentiation is performed on a fibronectin-coated surface.

21. A composition comprising a population of mesoderm-derived vascular progenitor cells (meso-VPCs) produced by the method of any one of claims 1-19, wherein the population of meso-VPCs expresses at least one cell-surface marker selected from the group consisting of CD31/PECAM1, CD309/KDR, CD43, CD144, CD34, CD184/CXCR4, CD146, and PDGFRb.

22. The composition of claim 21, wherein the population of meso-VPCs

(i) expresses at least two cell-surface markers selected from the group consisting of CD31/PECAM1, CD309/KDR, CD43, CD144, CD34, CD184/CXCR4, CD146, and PDGFRb;

(ii) expresses cell surface markers CD146, CD31/PECAM1, and CD309/KDR;

(iii) expresses cell surface markers CD31/PECAM1, CD309/KDR, CD146, and (i) at least one of CD144, CD34, CD184/CXCR4, CD43, or PDGFRb, (ii) CD34, CD184/CXCR4, and PDGFRb, (iii) CD184/CXCR4, (iv) PDGFRb; (v) CD144 and CD184/CXCR4, (vi) CD184/CXCR4 and CD43, or (vii) CC184/CXCFR4;

(iv) exhibits limited or no detection of (a) one or more cell surface markers selected from the group consisting of CXCR7, CD45, and NG2; or (b) CXCR7, CD45, and NG2;

(v) expresses at least one miRNA marker selected from hsa-miR-3917, hsa-miR-450a-2-3p, hsa-miR-542-5p, hsa-miR-126-5p, hsa-miR-125a-5p, hsa-miR-24-3p, hsa-let-7e-5p, hsa-miR-99a-5p, hsa-miR-223-5p, hsa-miR-142-3p, hsa-miR-483-5p, hsa-miR-483-3p, miR 214, miR 335-3p, and miR-199a-3p;

(vi) exhibits limited or no expression of at least one miRNA marker selected from hsa-let-7e-3p, hsa-miR-99a-3p, hsa-miR-133a-5p, hsa-miR-11399, hsa-miR-196b-3p, hsa-miR-5690, and hsa-miR-7151-3p; and/or

(vii) expresses hsa-miR-3917, hsa-miR-450a-2-3p, and hsa-miR-542-5p;

(viii) expresses at least one miRNA markers selected from the group consisting of mir126, mir125a-5p, mir24, and mir483-5p; and/or

(ix) exhibits limited or no expression for at least one miRNA markers selected from the group consisting of mir367, mir302a, mir302b, mir302c, mirLet7-e, mir223, mir99a, mir142-3p, and mir133a.

23. The composition of claim 21 or 22, wherein the population of meso-VPCs comprises (a) vasculonoids of meso-VPCs, or (b) single cells of meso-VPCs.

24. A composition comprising a population of meso-VPC produced by the method of any one of claims 1-19, wherein the meso-VPC is positive for at least one miRNA marker selected from the group consisting of mir126, mir125a-5p, mir24, and mir483-5p.

25. The composition of claim 24, wherein the meso-VPC is

(i) positive for miRNA marker mir483-5p and/or

(ii) negative for at least one miRNA marker selected from the group consisting of mir367, mir302a, mir302b, mir302c, mirLet7-e, mir223, mir99a, mir142-3p, and mir133a.

26. The composition of any one of claims 22-25, wherein the pluripotent stem cell is a human pluripotent stem cell, optionally, wherein the pluripotent stem cell is human embryonic stem cell (hESC) or human induced pluripotent stem cell (hiPSC).

27. The composition of any one of claims 22-26, wherein the pluripotent stem cell is first differentiated into a mesoderm cell which, in turn, is differentiated into the meso-VPC.

28. A pharmaceutical composition comprising the composition of any one of claims 22-27.

29. Use of the composition of any one of claims 22-27 or the pharmaceutical composition of claim 28 in the manufacture of a medicament for treating a vascular disease or disorder in a subject.

30. The use of claim 29, wherein the vascular disease or disorder is selected from the group consisting of atherosclerosis, peripheral artery disease (PAD), carotid artery disease, venous disease, blood clots, aortic aneurysm, fibromuscular dysplasia, lymphedema, and vascular injury, optionally, wherein the peripheral artery disease is selected from the group consisting of critical limb ischemia, intestinal ischemic syndrome, renal artery disease, popliteal entrapment syndrome, Raynaud's phenomenon, Buerger's disease.

31. The use of claim 29 or 30, wherein the composition or the pharmaceutical composition is to be administered intramuscularly or systemically.

32. The use of any one of claim 31, wherein the administration of the composition or the pharmaceutical composition

(a) increases the blood flow in the subject;

(b) promotes the angiogenesis and/or vasculogenesis in the subject;

(c) reduces the ischemic severity in the subject, and/or

(d) reduces the necrosis area of the limb in the subject.

33. The use of any one of claims 29-32, wherein the composition or the pharmaceutical composition comprise $1x10^4$ to $1x10^{13}$ meso-VPCs.

34. The use of any one of claims 29-33, wherein the pharmaceutical composition comprises

(a) a buffer, maintaining the solution at a physiological pH;

(b) at least 5% (w/v) glucose; optionally, wherein the glucose is D-glucose (Dextrose); and

(c) an osmotically active agent maintaining the solution at a physiologically osmolality; wherein the osmotically active agent is a salt, optionally, wherein the salt is sodium chloride.