Summary ~~of the Invention~~

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

[008] Accordingly, in one aspect, the present ~~invention~~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 ~~embodiment~~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 ~~embodiment~~example, the meso-VPCS are produced as a vasculonoid. In another ~~embodiment~~example, the meso-VPCs are dissociated into single cells.

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

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

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

concentration of about 2-10 μM. In one ~~embodiment~~example, the VEGF165 is used at a concentration of about 10-50 ng/mL. In one ~~embodiment~~example, the FGF-2 is used at a concentration of about 10-50 ng/mL. In one ~~embodiment~~example, the BMP4 is used at a concentration of about 10-50 ng/mL. In one ~~embodiment~~example, the SB431542 is used at a concentration of about 5-20 μM.

[014] In one ~~embodiment~~example, culturing the mesoderm cell is performed for about 3 days to about 7 days.

[015] In one ~~embodiment~~example, culturing the mesoderm cell is conducted under a normoxia condition of 5% CO_2 and 20% O_2.

[016] In one ~~embodiment~~example, culturing of the pluripotent stem cells is conducted under a normoxia condition of 5% CO_2 and 20% O_2.

[017] In one ~~embodiment~~example, the non-adherent or low adherent conditions are on an ultra-low attachment surface.

[018] In one aspect, the present ~~invention~~disclosure provides a method of producing a population of mesoderm-derived vascular progenitor cell (meso-VPC) from a pluripotent stem cell, wherein the method comprises (a) culturing a mesoderm cell derived from a pluripotent stem cell on an extracellular matrix surface, in a medium comprising one or more factors selected from the group consisting of vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and bone morphogenetic protein 4 (BMP4); and (b) culturing the cells produced in step (a) on an extracellular matrix surface, 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 the population of mesoderm-derived vascular progenitor cells.

[019] In one ~~embodiment~~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).

[020] In one ~~embodiment~~example, the method further comprises dissociating the population of meso-VPCs into single cells.

[021] In one ~~embodiment~~example, the mesoderm inducing growth factors comprise Activin-A, VEGF165, FGF-2 and BMP4. In one ~~embodiment~~example, the Activin-A is used at a concentration of about 5-15 ng/mL. In one ~~embodiment~~example, the VEGF165 is used at a concentration of about 5-25 ng/mL. In one ~~embodiment~~example, the FGF-2 is used at a concentration of about 5-25 ng/mL. In one ~~embodiment~~example, the BMP4 is used at a concentration of about 5-50 ng/mL. In one ~~embodiment~~example,

the method further comprises removing Activin-A from the culture media after about 24 hours of culturing.

[022] In one ~~embodiment~~example, the extracellular matrix surface in step (a) is a collagen IV-coated surface.

[023] In one ~~embodiment~~example, the pluripotent stem cells are cultured for about 3 days to about 5 days.

[024] In one ~~embodiment~~example, the one or more factors in step (a) comprise VEGF165, FGF-2, and BMP4.

[025] In one ~~embodiment~~example, the small molecule inhibitor of transforming growth factor-beta (TGF-β) type I receptor is SB431542. In another ~~embodiment~~example, the one or more factors in step (b) comprise VEGF165, FGF-2, BMP4, and SB431542.

[026] In one ~~embodiment~~example, the one or more factors in step (a) further comprises Forskolin.

[027] In one ~~embodiment~~example, the one or more factors in step (b) further comprises Forskolin.

[028] In one ~~embodiment~~example, the Forskolin is used at a concentration of about 2-10 μM.

[029] In one ~~embodiment~~example, the VEGF165 is used at a concentration of about 10-50 ng/mL.

[030] In one ~~embodiment~~example, the FGF-2 is used at a concentration of about 10-50 ng/mL.

[031] In one ~~embodiment~~example, the BMP4 is used at a concentration of about 10-50 ng/mL.

[032] In one ~~embodiment~~example, the SB431542 is used at a concentration of about 5-20 μM.

[033] In one ~~embodiment~~example, the extracellular matrix surface in steps (a) and (b) is a collagen-IV-coated surface.

[034] In one ~~embodiment~~example, the culturing in step (a) is performed for about 1 day.

[035] In one ~~embodiment~~example, the culturing in step (b) is performed for about 4 days to about 7 days.

[036] In one ~~embodiment~~example, the culturing in step (a) is conducted under a normoxia condition of 5% CO_2 and 20% O_2.

[037] In one ~~embodiment~~example, the culturing in step (b) is conducted under a hypoxia condition of 5% CO_2 and 5% O_2.

[038] In one ~~embodiment~~example, culturing of the pluripotent stem cells is conducted under a normoxia condition of 5% CO_2 and 20% O_2.

[039] In one ~~embodiment~~example, the pluripotent stem cell is a human embryonic stem cell.

[040] In one ~~embodiment~~example, the pluripotent stem cell is a human induced pluripotent stem cell.

[041] In one ~~embodiment~~example, the population of meso-VPCs produced according to any of the methods of the present ~~invention~~disclosure 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.

[042] In one ~~embodiment~~example, the population of meso-VPCs produced according to any of the methods of the present ~~invention~~disclosure expresses cell-surface markers (a) CD146, CD31/PECAM1, and CD309/KDR;.or (b) 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.

[043] In one ~~embodiment~~example, the population of meso-VPCs produced according to any of the methods of the present ~~invention~~disclosure exhibits limited or no detection of (a) one or more of cell-surface markers selected from the group consisting of CXCR7, CD45, and NG2; (b) CXCR7, CD45, and NG2; or (c) one or more of cell-surface markers selected from the group consisting of CD144, CD34, CD184/CXCR4, CXCR7, CD43, CD45, PDGFRb, and NG2.

[044] In one ~~embodiment~~example, the population of meso-VPCs produced according to any of the methods of the present ~~invention~~disclosure 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.

[045] In one ~~embodiment~~example, the population of meso-VPCs produced according to any of the methods of the present ~~invention~~disclosure 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.

[046] In one ~~embodiment~~example, the population of meso-VPCs produced according to any of the methods of the present ~~invention~~disclosure expresses hsa-miR-3917, hsa-miR-450a-2-3p, and hsa-miR-542-5p.

[047] In one ~~embodiment~~example, the population of meso-VPCs produced according to any of the methods of the present ~~invention~~disclosure 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. In one ~~embodiment~~example, the miRNA marker is mir483-5p.

[048] In one ~~embodiment~~example, the population of meso-VPCs produced according to any of the methods of the present ~~invention~~disclosure 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.

[049] In one ~~embodiment~~example, the methods of the present ~~invention~~disclosure further comprise producing a vascular endothelial cell by differentiation of the meso-VPC.

[050] In one ~~embodiment~~example, the differentiation is performed on a fibronectin-coated surface.

[051] In one aspect, the present ~~invention~~disclosure provides a composition comprising a population of meso-VPCs produced by any one of the methods of the ~~invention~~disclosure.

[052] In one aspect, the present ~~invention~~disclosure provides a composition comprising a population of mesoderm-derived vascular progenitor cells (meso-VPCs) produced by *in vitro* differentiation of a mesoderm cell derived from a pluripotent stem cell, 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.

[053] In one ~~embodiment~~example, the composition comprising a population of meso-VPCs 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.

[054] In one ~~embodiment~~example, the composition comprising a population of meso-VPCs expresses cell surface markers CD146, CD31/PECAM1, and CD309/KDR.

[055] In one ~~embodiment~~example, the composition comprising a population of meso-VPCs 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.

[056] In one ~~embodiment~~example, the composition comprising a population of meso-VPCs exhibits limited or no detection of (a) one or more cell surface markers selected from the group consisting of CXCR7, CD45, and NG2; (b) CXCR7, CD45, and NG2; or (c) one or more cell surface markers selected from the group consisting of CD144, CD34, CD184/CXCR4, CXCR7, CD43, CD45, PDGFRb, and NG2.

[057] In one ~~embodiment~~example, the composition comprising a population of meso-VPCs 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.

[058] In one ~~embodiment~~example, the composition comprising a population of meso-VPCs 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.

[059] In one ~~embodiment~~example, the composition comprising a population of meso-VPCs expresses hsa-miR-3917, hsa-miR-450a-2-3p, and hsa-miR-542-5p.

[060] In one ~~embodiment~~example, the population of meso-VPCs comprises vasculonoids of meso-VPCs.

[061] In one ~~embodiment~~example, the population of meso-VPCs comprises single cells of meso-VPCs.

[062] In one ~~embodiment~~example, the present ~~invention~~disclosure provides a meso-VPC produced by in vitro differentiation of a mesoderm cell derived from a pluripotent stem cell, 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.

[063] In one ~~embodiment~~example, the meso-VPC is positive for miRNA marker mir483-5p.

[064] In one ~~embodiment~~example, the meso-VPC is 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.

[065] In one ~~embodiment~~example, the pluripotent stem cell is a human pluripotent stem cell.

[066] In one ~~embodiment~~example, the pluripotent stem cell is human embryonic stem cell (hESC).

[067] In one ~~embodiment~~example, the pluripotent stem cell is human induced pluripotent stem cell (hiPSC).

[068] In one ~~embodiment~~example, the pluripotent stem cell is first differentiated into a mesoderm cell which, in turn, is differentiated into the meso-VPC.

[069] In one aspect, the present disclosure provides a pharmaceutical composition comprising a composition as disclosed herein. In one example, the present ~~invention~~disclosure provides a pharmaceutical composition comprising a composition comprising a population of meso-VPCs or any one of the meso-VPCs of the present ~~invention~~disclosure.

[070] In one aspect, the present disclosure provides the use of the composition as disclosed herein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a vascular disease or disorder in a subject. In one example, the present ~~invention~~disclosure provides a method of treating a vascular disease or disorder in a subject, the method comprising administering to the subject an effective amount of any one of the compositions comprising a population of meso-VPCs or mesoderm-derived vascular progenitor cells (meso-VPCs) of the present ~~invention~~disclosure, or any one of the pharmaceutical compositions of the present ~~invention~~disclosure, thereby treating the vascular disease or disorder in the subject.

[071] In one ~~embodiment~~example, 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.

[072] In one ~~embodiment~~example, 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.

[073] In one ~~embodiment~~example, the periphery artery disease is critical limb ischemia.

[074] In one ~~embodiment~~example, the composition comprising a population of meso-VPCs, meso-VPC, or the pharmaceutical composition is administered intramuscularly or systemically.

[075] In one ~~embodiment~~example, the administration of the composition comprising a population of meso-VPCs, meso-VPC, or the pharmaceutical composition increases the blood flow in the subject.

[076] In one ~~embodiment~~example, the administration of the composition comprising a population of meso-VPCs, meso-VPC, or the pharmaceutical composition promotes the angiogenesis and/or vasculogenesis in the subject.

[077] In one ~~embodiment~~example, the administration of the composition comprising a population of meso-VPCs, meso-VPC, or the pharmaceutical composition reduces the ischemic severity in the subject.

[078] In one ~~embodiment~~example, the administration of the composition comprising a population of meso-VPCs, meso-VPC, or the pharmaceutical composition reduces the necrosis area of the limb in the subject.

[079] In one ~~embodiment~~example, about $1x10^4$ to about $1x10^{13}$ meso-VPCs are administered to the subject.

[080] In one ~~embodiment~~example, the meso-VPC is administered in a pharmaceutical composition.

[081] In one ~~embodiment~~example, the pharmaceutical composition comprises (a) a buffer, maintaining the solution at a physiological pH; (b) at least 5% (w/v) glucose; and (c) an osmotically active agent maintaining the solution at a physiologically osmolality.

[082] In one ~~embodiment~~example, the glucose is D-glucose (Dextrose).

[083] In one ~~embodiment~~example, the osmotically active agent is a salt.

[084] In one ~~embodiment~~example, the salt is sodium chloride.

Brief Description of the Drawings

[085] FIG. 1 is a schematic illustration of the process for the in vitro differentiation of human pluripotent stem cells into mesoderm cells.

Claims

1. 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).

2. The method of claim 1, wherein 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).

3. The method of claim 1 or 2, 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 3, further comprising dissociating the meso-VPCs in the vasculonoid into single cells.~~

~~5.~~4. The method of ~~any one of~~ claim~~s~~ 2 ~~to 4~~, wherein the mesoderm inducing growth factors comprise Activin-A, VEGF165, FGF-2 and BMP4, optionally, wherein the Activin-A is used at a concentration of about 5-15 ng/mL, the VEGF165 is used at a concentration of about 5-25 ng/mL, the FGF-2 is used at a concentration of about 5-25 ng/mL, and/or the BMP4 is used at a concentration of about 5-50 ng/mL.

~~6. The method of claim 5, wherein the Activin-A is used at a concentration of about 5-15 ng/mL.~~

~~7. The method of claim 5, wherein the VEGF165 is used at a concentration of about 5-25 ng/mL.~~

~~8. The method of claim 5, wherein the FGF-2 is used at a concentration of about 5-25 ng/mL.~~

~~9. The method of claim 5, wherein the BMP4 is used at a concentration of about 5-50 ng/mL.~~

~~10.~~5. The method of any one of claims 2-~~9~~4, further comprising removing Activin-A from the culture media after about 24 hours of culturing.

~~11.~~6. The method of any one of claims 2-~~10~~5, wherein (a) the pluripotent stem cells are cultured on an extracellular matrix surface, optionally wherein the extracellular matrix surface is a Matrigel-coated surface; and/or (b) the pluripotent stem cells are cultured for 3 days to 5 days.

~~12. The method of claim 11, wherein the extracellular matrix surface is a Matrigel-coated surface.~~

~~13. The method of any one of claims 2-12, wherein the pluripotent stem cells are cultured for about 3 days to about 5 days.~~

~~14.~~7. The method of any one of claims 1-~~13~~6, wherein (i) the small molecule inhibitor of transforming growth factor-beta (TGF-β) type I receptor is SB431542; (ii) the one or more factors in step (a) comprise VEGF165, FGF-2, BMP4 and SB431542; optionally, wherein the VEGF165 is used at a concentration of about 10-50 ng/mL, the FGF-2 is used at a concentration of about 10-50 ng/mL, the BMP4 is used at a concentration of about 10-50 ng/mL, and/or the SB431542 is used at a concentration of about 5-20 µM; and/or (iii) the one or more factors further comprises Forskolin, optionally, wherein the Forskolin is used at a concentration of about 2-10 µM.

~~15. The method of any one of claims 1-14, wherein the one or more factors comprise VEGF165, FGF-2, BMP4, and SB431542.~~

~~16. The method of any one of claims 1-15, wherein the one or more factors further comprises Forskolin.~~

~~17. The method of claim 16, wherein the Forskolin is used at a concentration of about 2-10 µM.~~

~~18. The method of any one of claims 15-17, wherein the VEGF165 is used at a concentration of about 10-50 ng/mL.~~

~~19. The method of any one of claims 15-17, wherein the FGF-2 is used at a concentration of about 10-50 ng/mL.~~

~~20. The method of any one of claims 15-17, wherein the BMP4 is used at a concentration of about 10-50 ng/mL.~~

~~21. The method of any one of claims 14-20, wherein the SB431542 is used at a concentration of about 5-20 µM.~~

~~22.~~8. The method of any one of claims 1-~~21~~7, wherein culturing the mesoderm cell is (a) performed for ~~about~~ 3 days to ~~about~~ 7 days; (b) conducted under a normoxia condition of 5% CO_2 and 20% O_2; and/or (c) conducted under a normoxia condition of 5% CO_2 and 20% O_2.

~~23. The method of any one of claims 1-22, wherein culturing the mesoderm cell is conducted under a normoxia condition of 5% CO_2 and 20% O_2.~~

~~24. The method of any one of claims 2-23, wherein culturing of the pluripotent stem cells is conducted under a normoxia condition of 5% CO_2 and 20% O_2.~~

~~25.~~9. The method of any one of claims 1-~~24~~8, wherein the non-adherent or low adherent conditions are on an ultra-low attachment surface.

~~26.~~10. A method of producing a population of mesoderm-derived vascular progenitor cell (meso-VPC) from a pluripotent stem cell, wherein the method comprises

(a) culturing a mesoderm cell derived from a pluripotent stem cell on an extracellular matrix surface, in a medium comprising one or more factors selected from the group consisting of vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and bone morphogenetic protein 4 (BMP4); and

(b) culturing the cells produced in step (a) on an extracellular matrix surface, 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 the population of mesoderm-derived vascular progenitor cells.

~~27.~~11. The method of claim ~~26~~10, wherein 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).

~~28.~~12. The method of claim ~~26~~ 10 or ~~27~~11, wherein the method further comprises dissociating the population of meso-VPCs into single cells.

~~29.~~13. The method of claim ~~27~~ 11 or ~~28~~12, wherein the mesoderm inducing growth factors comprise Activin-A, VEGF165, FGF-2 and BMP4, optionally, wherein the Activin-A is used at a concentration of about 5-15 ng/mL, the VEGF165 is used at a concentration of about 5-25 ng/mL, the FGF-2 is used at a concentration of about 5-25 ng/mL, and/or the BMP4 is used at a concentration of about 5-50 ng/mL.

~~30. The method of claim 29, wherein the Activin-A is used at a concentration of about 5-15 ng/mL.~~

~~31. The method of claim 29, wherein the VEGF165 is used at a concentration of about 5-25 ng/mL.~~

~~32. The method of claim 29, wherein the FGF-2 is used at a concentration of about 5-25 ng/mL.~~

~~33. The method of claim 29, wherein the BMP4 is used at a concentration of about 5-50 ng/mL.~~

~~34.~~14. The method of any one of claims ~~29-33~~13, further comprising removing Activin-A from the culture media after about 24 hours of culturing.

~~35.~~15. The method of any one of claims ~~26-34~~10-14, wherein (a) the extracellular matrix surface in step (a) is a collagen IV-coated surface; and/or (b) the pluripotent stem cells are cultured for 3 days to 5 days.

~~36. The method of any one of claims 27-35, wherein the pluripotent stem cells are cultured for about 3 days to about 5 days.~~

~~37.~~16. The method of any one of claims ~~26-36~~10-16, wherein (i) the small molecule inhibitor of transforming growth factor-beta (TGF-β) type I receptor is SB431542; optionally, wherein the SB431542 is used at a concentration of about 5-20 µM; (ii) the one or more factors in step (a) comprise VEGF165, FGF-2, and BMP4; optionally, wherein the VEGF165 is used at a concentration of about 10-50 ng/mL, the FGF-2 is used at a concentration of about 10-50 ng/mL and/or the BMP4 is used at a concentration of about 10-50 ng/mL; (iii) the one or more factors in step (b) comprise VEGF165, FGF-2, BMP4 and SB431542; and/or (iv) the one or more factors in step (a) and/or step (b) further comprises Forskolin, optionally, wherein the Forskolin is used at a concentration of about 2-10 µM.

~~38. The method of any one of claims 26-37, wherein the small molecule inhibitor of transforming growth factor-beta (TGF-β) type I receptor is SB431542.~~

~~39. The method of any one of claims 26-38, wherein the one or more factors in step (b) comprise VEGF165, FGF-2, BMP4, and SB431542.~~

~~40. The method of any one of claims 26-39, wherein the one or more factors in step (a) further comprises Forskolin.~~

~~41. The method of claim any one of claims 26-40, wherein the one or more factors in step (b) further comprises Forskolin.~~

~~42. The method of claim 40 or 41, wherein the Forskolin is used at a concentration of about 2-10 µM.~~

~~43. The method of any one of claims 37-42, wherein the VEGF165 is used at a concentration of about 10-50 ng/mL.~~

~~44. The method of any one of claims 37-42, wherein the FGF-2 is used at a concentration of about 10-50 ng/mL.~~

~~45. The method of any one of claims 37-42, wherein the BMP4 is used at a concentration of about 10-50 ng/mL.~~

~~46. The method of claims 38 or 39, wherein the SB431542 is used at a concentration of about 5-20 µM.~~

~~47.~~17. The method of any one of claims ~~26-46~~10-16, wherein (i) the extracellular matrix surface in steps (a) and (b) is a collagen-IV-coated surface~~;~~.

(ii) the culturing in step (a) is performed for about 1 day;

(iii) the culturing in step (b) is performed for 4 days to 7 days;

(iv) the culturing in step (a) is conducted under a normoxia condition of 5% CO_2 and 20% O_2,

(v) the culturing in step (b) is conducted under a hypoxia condition of 5% CO_2 and 5% O_2, and/or

(vi) culturing of the pluripotent stem cells is conducted under a normoxia condition of 5% CO_2 and 20% O_2.

~~48. The method of any one of claims 26-47, wherein the culturing in step (a) is performed for about 1 day.~~

~~49. The method of any one of claims 26-48, wherein the culturing in step (b) is performed for about 4 days to about 7 days.~~

~~50. The method of any one of claims 26-49, wherein the culturing in step (a) is conducted under a normoxia condition of 5% CO_2 and 20% O_2.~~

~~51. The method of any one of claims 26-50, wherein the culturing in step (b) is conducted under a hypoxia condition of 5% CO_2 and 5% O_2.~~

~~52. The method of any one of claims 27-51, wherein culturing of the pluripotent stem cells is conducted under a normoxia condition of 5% CO_2 and 20% O_2.~~

~~53.~~18. The method of any one of claims 1-~~52~~17, wherein the pluripotent stem cell is a human embryonic stem cell or a human induced pluripotent stem cell.

~~54. The method of any one of claims 1-52, wherein the pluripotent stem cell is a human induced pluripotent stem cell.~~

~~55.~~19. The method of any one of claims 1-~~54~~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; (b) CXCR7, CD45, and NG2; or (c) one or more of cell-surface markers selected from the group consisting of CD144, CD34, CD184/CXCR4, CXCR7, CD43, CD45, PDGFRb, 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.

~~56. The method of claim 55, wherein the population of meso-VPC expresses cell-surface markers (a) CD146, CD31/PECAM1, and CD309/KDR;.or (b) 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.~~

~~57. The method of any one of claims 1-56, wherein the population of meso-VPC exhibits limited or no detection of (a) one or more of cell-surface markers selected from the group consisting of CXCR7, CD45, and NG2; (b) CXCR7, CD45, and NG2; or (c) one or more of cell-surface markers selected from the group consisting of CD144, CD34, CD184/CXCR4, CXCR7, CD43, CD45, PDGFRb, and NG2.~~

~~58. The method of any one of claims 1-57, wherein the population of meso-VPCs 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.~~

~~59. The method of any one of claims 1-58, wherein the population of meso-VPCs 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.~~

~~60. The method of any one of claims 1-59, wherein the population of meso-VPCs expresses hsa-miR-3917, hsa-miR-450a-2-3p, and hsa-miR-542-5p.~~

~~61. The method of any one of claims 1-60, wherein the population of meso-VPCs 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.~~

~~62. The method of claim 61, wherein the miRNA marker is mir483-5p.~~

~~63. The method of any one claims 1-62, wherein the population of meso-VPCs 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.~~

~~64.~~20. The method of any one of claims 1-~~63~~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.

~~65. The method of claim 64, wherein the differentiation is performed on a fibronectin-coated surface.~~

~~66.~~21. A composition comprising a population of meso-VPC produced by the method of any one of claims 1-~~63~~19.

~~67.~~22. A composition comprising a population of mesoderm-derived vascular progenitor cells (meso-VPCs) produced by *in vitro* differentiation of a mesoderm cell derived from a pluripotent stem cell, 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.

~~68.~~23. The composition of claim ~~67~~22, 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; (b) CXCR7, CD45, and NG2; or (c) one or more cell surface markers selected from the group consisting of CD144, CD34, CD184/CXCR4, CXCR7, CD43, CD45, PDGFRb, 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.

~~69. The composition of any one of claims 67-68, wherein the population of meso-VPCs expresses cell surface markers CD146, CD31/PECAM1, and CD309/KDR.~~

~~70. The composition of any one of claims 67-69, wherein the population of meso-VPC 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.~~

~~71. The composition of any one of claims 67-70, wherein the population of meso-VPCs exhibits limited or no detection of (a) one or more cell surface markers selected from the group consisting of CXCR7, CD45, and NG2; (b) CXCR7, CD45, and NG2; or (c) one or more cell surface markers selected from the group consisting of CD144, CD34, CD184/CXCR4, CXCR7, CD43, CD45, PDGFRb, and NG2.~~

~~72. The composition of any one of claims 67-71, wherein the population of meso-VPCs 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.~~

~~73. The composition of any one of claims 67-72, wherein the population of meso-VPCs 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.~~

~~74. The composition of any one of claims 67-73, wherein the population of meso-VPCs expresses hsa-miR-3917, hsa-miR-450a-2-3p, and hsa-miR-542-5p.~~

~~75.~~24. The composition of ~~any one of~~ claim~~s 67-74~~22 or 23, wherein the population of meso-VPCs comprises (a) vasculonoids of meso-VPCs, or (b) single cells of meso-VPCs.

~~76. The composition of any one of claims 67-74, wherein the population of meso-VPCs comprise single cells of meso-VPCs.~~

~~77.~~25. A composition comprising a population of meso-VPC produced by *in vitro* differentiation of a mesoderm cell derived from a pluripotent stem cell, 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.

~~78.~~26. The ~~meso-VPC~~composition of claim ~~77~~25, 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.

~~79. The meso-VPC of claim 77 or 78, wherein the meso-VPC is 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.~~

~~80.~~27. The composition ~~or meso-VPC~~ of any one of claims ~~66-79~~21-26, 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).

~~81. The composition or meso-VPC of claim 80, wherein the pluripotent stem cell is human embryonic stem cell (hESC).~~

~~82. The composition or meso-VPC of claim 80, wherein the pluripotent stem cell is human induced pluripotent stem cell (hiPSC).~~

~~83.~~28. The composition ~~or meso-VPC~~ of any one of claims ~~66-82~~21-27, wherein the pluripotent stem cell is first differentiated into a mesoderm cell which, in turn, is differentiated into the meso-VPC.

~~84.~~29. A pharmaceutical composition comprising the composition ~~or meso-VPC~~ of any one of claims ~~66-83~~21-28.

~~85.~~30. Use of the composition of any one of claims 21-28 or the pharmaceutical composition of claim 29 in the manufacture of a medicament for~~A method of~~ treating a vascular disease or disorder in a subject~~, the method comprising administering to the subject an effective amount of a composition or mesoderm-derived vascular progenitor cells~~

~~(meso-VPCs) of any one of claims 66-83, or the pharmaceutical composition of claim 83, thereby treating the vascular disease or disorder in the subject~~.

~~86.~~31. The ~~method~~use of claim ~~85~~30, 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.

~~87. The method of claim 86, 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.~~

~~88. The method of claim 87, wherein the periphery artery disease is critical limb ischemia.~~

~~89.~~32. The ~~method~~ use of ~~any one of~~ claim~~s 85-88~~30 or 31, wherein the composition~~, meso-VPC,~~ or the pharmaceutical composition is to be administered intramuscularly or systemically.

~~90.~~33. The ~~method~~ use of any one of claims ~~85-89~~30-32, wherein the administration of the composition~~, meso-VPC,~~ 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.

~~91. The method of any one of claims 85-90, wherein the administration of the composition, meso-VPC, or the pharmaceutical composition promotes the angiogenesis and/or vasculogenesis in the subject.~~

~~92. The method of any one of claims 85-91, wherein the administration of the composition, meso-VPC, or the pharmaceutical composition reduces the ischemic severity in the subject.~~

~~93. The method of any one of claims 85-92, wherein the administration of the composition, meso-VPC, or the pharmaceutical composition reduces the necrosis area of the limb in the subject.~~

~~94.~~34. The ~~method~~ use of any one of claims ~~85-93~~30-33, wherein about $1x10^4$ to about $1x10^{13}$ meso-VPCs are to be administered to the subject.

~~95.~~35. The ~~method~~ use of any one of claims ~~85-94~~30-34, wherein the meso-VPC is to be administered in a pharmaceutical composition; 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.

~~96. The method of claim 95, wherein the pharmaceutical composition comprises~~

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

~~(b) at least 5% (w/v) glucose; and~~

~~(c) an osmotically active agent maintaining the solution at a physiologically osmolality.~~

~~97. The method of claim 96, wherein the glucose is D-glucose (Dextrose).~~

~~98. The method of claim 96, wherein the osmotically active agent is a salt.~~

~~99. The method of claim 96, wherein the salt is sodium chloride.~~