K. Claims

What is claimed:

1. An isolated PVMC.

2. The isolated PVMC of claim 1, wherein the PVMC is derived from bone.

3. The isolated PVMC of claim 2, wherein the bone comprises a bone chip, a trabecular bone cavity, bone marrow, bone cavity lavage, or a combination thereof.

4. The PVMC of claim 1, wherein the PVMC is derived from an umbilical cord blood vessel.

5. A method of producing bone chips comprising passing a bone fragment through a grinder or bone mill.

6. The method of claim 5, wherein the bone fragment is cryogenically frozen

7. A method of separating osteogenic cells from a PVMC preparation comprising determining adsorption of a cell in the preparation to calcium phosphate substrates, wherein a high affinity indicates the presence of an osteogenic cell.

8. A method of making an enhanced, autologous bone graft comprising (i) extracting from a subject a first portion of bone tissue to be used as a bone graft, then (ii) supplementing the bone graft with a population of concentrated PVMCs.

9. A method for making an enhanced, autologous bone graft comprising:

a. extracting a cell suspension from a first portion of bone tissue from a subject with an enzyme, mechanical force, or a combination thereof;

b. concentrating the cells in the cell suspension by buoyant density sedimentation, filtration or centrifugation to obtain a population of concentrated bone-derived PVMCs; and

c. supplementing a second portion of bone tissue to be used as a bone graft from the subject with the population of concentrated bone-derived PVMCs, so as to make the enhanced, autologous bone graft.

10. The method of claim 9, wherein the first portion of bone tissue originates from the proximal region of a femur, the distal region of a femur, or a combination thereof.

11. The method of claim 9, wherein the second portion of bone tissue originates from human bones comprising at least one of an ilium crest, a femur, a patella, a tibia, a humerus, a clavicle, a rib, a scapula, or a combination thereof.

12. The method of claim 9, further comprising, supplementing the enhanced, autologous bone graft with fresh autologous bone marrow, processed autologous bone marrow, frozen autologous bone marrow, fresh autologous bone, processed autologous bone, frozen autologous bone, or a combination thereof.

13. A pharmaceutical composition comprising a therapeutically effective amount of a plurality of isolated PVMCs and a pharmaceutically acceptable carrier.

14. The pharmaceutical composition of claim 13, wherein the plurality of PVMCs comprises PVMCs derived from bone, an umbilical cord blood vessel, an anatomic source containing PVMCs, or a combination thereof.

15. The pharmaceutical composition of claim 14, wherein the bone comprises a bone chip, a trabecular bone cavity, bone marrow, bone cavity lavage, or a combination thereof.

16. The pharmaceutical composition of claim 13, further comprising bone marrow cells.

17. The pharmaceutical composition of claim 13, further comprising a scaffold material.

18. The pharmaceutical composition of claim 17, wherein the scaffold material comprises bone chips, ceramic-based bone graft substitutes, calcium phosphate ceramics, calcium sulfate ceramics, bioglass, polymer-based bone graft substitutes, degradable and nondegradable polymers, processed allograft bone material, mineralized

processed allograft, demineralized processed allograft, collagen sponges, or combinations thereof.

19. A method of modulating apoptosis comprising administering a composition comprising a therapeutically effective amount of PVMCs to a subject in need thereof.

20. A method of modulating mitosis comprising administering a composition comprising a therapeutically effective amount of PVMCs to a subject in need thereof.

21. A method of modulating angiogenesis comprising administering a composition comprising a therapeutically effective amount of PVMCs to a subject in need thereof.

22. A method of modulating bone formation comprising administering a composition comprising a therapeutically effective amount of PVMCs to a subject in need thereof.

23. The method of claim 22, wherein the perivascular medicinal cells have the capability to form osteoblasts.

24. A method of immunomodulation comprising administering a composition comprising a therapeutically effective amount of PVMCs to a subject in need thereof.

25. A composition comprising a plurality of PVMCs and an acceptable carrier.

26. The composition of claim 25, wherein the plurality of PVMCs comprises PVMCs derived from bone, an umbilical cord blood vessel, or a combination thereof.

27. The composition of claim 26, wherein the bone comprises bone chips, bone marrow tissue and other tissue, compact bone, bone marrow from an intermedullary canal, a bone chip, a trabecular bone cavity, a bone cavity lavage, or combinations thereof.

28. The composition of claim 25, further comprising bone marrow cells.

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29. The composition of claim 25, further comprising a scaffold material.

30. The composition of claim 29, wherein the scaffold material comprises bone chips, ceramic-based bone graft substitutes, calcium phosphate ceramics, calcium sulfate ceramics, bioglass, polymer-based bone graft substitutes, degradable and nondegradable polymers, processed allograft bone material, mineralized processed allograft, demineralized processed allograft, collagen sponges, or combinations thereof.

31. A method for isolating PVMCs from bone the method comprising: (i) providing a sample of bone tissue from a subject; (ii) extracting the PVMCs from the bone; and (iii) concentrating the extracted PVMCs.

32. The method claim 31, further comprising grinding the bone tissue.

33. The method of claim 31, wherein extracting the PVMCs comprises: (i) extracting a cell suspension from the bone tissue by enzymatic digestion, mechanical force, or a combination thereof; and (ii) separating a population of PVMCs from the cell suspension by buoyant density sedimentation, filtration, centrifugation, or a combination thereof.

34. The method of claim 33, wherein the enzymatic digestion uses one or more enzymes that cleave the attachment of a PVMC from a basement membrane of a small blood vessel.

35. The method of claim 31, wherein concentrating the extracted PVMCs is achieved by methods comprising the use of magnetic beads containing antibodies with affinity to cell surface antigens on the PVMC.

36. The method of claim 35, wherein the antibodies are selected from anti-CD146, anti-CD105, anti-CD166, anti-CD271, or a combination thereof.

37. A method of treating a disease that affects cellular function comprising administering a composition comprising a therapeutically effective amount of PVMCs to a subject in need thereof.

38. The method of claim 37, wherein the PVMCs are capable of secreting a site-dependent trophic factor.

39. The method of claim 38, wherein the site-dependent trophic factor is selected from prostaglandin E2 (PGE2), stromal-cell derived factor-1 (SDF-1Vascular

endothelial growth factor (VEGF), VEGF165, interleukin-1β (IL-β), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-12 (IL-12), interleukin-16 (IL-16),, hepatocyte growth factor (HGF), transforming growth factor beta (TGF-β), basic fibroblast growth factor (bFGF), granulocyte–macrophage colony-stimulating factor (GM-CSF), insulin-like growth factor 1 (IGF-1), indoleamine 2,3-dioxygenase (IDO), interleukin-10 (IL-10), human leukocyte antigen G (HLA-G), leukemia inhibitory factor (LIF), class II major histocompatibility complex (MHC), eotaxin, granulocyte colony stimulating factor (G-csf), regulated upon activation, normal T-cell expressed and secreted (RANTES), IL-1 receptor antagonist (IL-1ra), tumor necrosis factor- α, TNF-α, tumor necrosis factor- β (TNF- β), epithelial neutrophil-activating protein 78 (ENA-78), eotaxin, monocyte chemoattractant protein 1 (MCP-1), monocyte chemoattractant protein 3 (MCP-3), macrophage inflammatory protein-1 α (MIP-1 α), macrophage inflammatory protein-3 α (MIP-3 α), macrophage inflammatory protein-1 β (MIP-1 β), intercellular adhesion molecule-1 (ICAM-1), VCAM-1, granulocyte colony-stimulating factor (G-CSF), growth hormone, stem cell factor (SCF), thyroid-stimulating hormone (TSH), CD40 and CD40 ligand, placental growth factor (PlGF), eotaxin-3, fractalkine, epithelial neutrophil-activating protein 78 (ENA-78), Interferon-inducible T-cell alpha chemoattractant (i-TAC), growth regulated oncogene-alpha (GROα), growth regulated oncogene-beta (GROβ), Interferon-inducible protein-10 (IP-10), CD146, CD105, CD166, CD44, CD271, CD73, CD90, CD44, CD10, or a combination thereof.

40. The method of claim 37, wherein the disease is ischemic heart disease, burns, stroke, inflammatory bowel disease, Crohn's disease, rheumatoid arthritis, lupus, amyotrophic lateral sclerosis, spinal cord damage, polytrauma, bone fractures, diabetes, or combinations thereof.

41. A method of reconstructing bone tissue comprising administering a composition comprising a therapeutically effective amount of PVMCs to a subject in need thereof.

42. A method of anchoring a metal device within a bone comprising administering a composition comprising a therapeutically effective amount of PVMCs to a subject in need thereof.

43. The method of claim 42, wherein the metal device is anchored in a bone selected from cranial-facial bone, cranium, mandible, clavicule, scapula, sternum, ribs, humerus, ulna, radius, carpels, phalange, metacarpal, patella, fibula, femur, tibia, tarsal, metatarsal, sacrum, coxa or lumbar vertebrae.

44. A method for isolating PVMCs from an umbilical cord blood vessel comprising: draining the umbilical cord blood vessel; adding a first enzyme mixture to the umbilical cord blood vessel to disassociate the PVMC; adding a medium; and collecting a wash eluent after adding the medium, wherein the wash eluent comprises a cell suspension of cells selected from endothelial, subendothelial cells, and combinations thereof.

45. The method of claim 44, wherein the umbilical cord blood vessel is a vein or artery.

46. The method of claim 44, wherein the first enzymatic mixture comprises an enzyme selected from collagenases, neutral or acidic proteases, GAGases, metalloproteases clostripain, serine proteases, alkaline proteases, cysteine proteases, and combinations thereof.

47. The method of claim 44, wherein the first enzyme mixture further comprises a second agent selected from a medium, an antibiotic, and a combination thereof.

48. The method of claim 47, wherein the medium is selected from Tyrode's solution, lactated Ringer's Solution, acetated Ringer's solution, TRIS-buffered saline (TBS), Hank's balanced salt solution (HBSS), Earle's balanced salt solution (EBSS), Standard saline citrate (SSC), HEPES-buffered saline (HBS), Gey's balanced salt solution (GBSS), minimum essential medium Eagle alpha modification (α-MEM), phosphate buffered saline (PBS), and combinations thereof.

49. The method of claim 47, wherein the antibiotic is selected from streptomycin, gentamicin, fungizone, penicillinG, amphotericin B, and combinations thereof.

50. The method of claim 44, wherein draining the umbilical cord blood vessel comprises inserting a needle in to the umbilical cord blood vessel and flushing the umbilical cord blood vessel with a medium.

51. The method of claim 44, wherein the medium is selected from Tyrode's solution, lactated Ringer's Solution, acetated Ringer's solution, TRIS-buffered saline (TBS), Hank's balanced salt solution (HBSS), Earle's balanced salt solution (EBSS), Standard saline citrate (SSC), HEPES-buffered saline (HBS), Gey's balanced salt solution (GBSS), minimum essential medium Eagle alpha modification (α-MEM), phosphate buffered saline (PBS), and combinations thereof.

52. The method of claim 44, further comprising incubating the umbilical cord blood vessel with the first enzyme mixture at a temperature ranging from about 15 °C to about 38 °C.

53. The method of claim 52, wherein the umbilical cord blood vessel may be incubated with the first enzyme mixture for about 15 to about 60 minutes.

54. The method of claim 52, further comprising inactivating the enzyme.

55. The method of claim 44, wherein, the medium is selected from Tyrode's solution, lactated Ringer's Solution, acetated Ringer's solution, TRIS-buffered saline (TBS), Hank's balanced salt solution (HBSS), Earle's balanced salt solution (EBSS), Standard saline citrate (SSC), HEPES-buffered saline (HBS), Gey's balanced salt solution (GBSS), minimum essential medium Eagle alpha modification (α-MEM), phosphate buffered saline (PBS), or a combination thereof.

56. The method of claim 44, further comprising adding a second enzyme mixture to the umbilical cord blood vessel.

57. The method of claim 56, wherein, the second enzyme mixture comprises an enzyme selected from collagenases, neutral or acidic proteases, GAGases, metalloproteases clostripain, serine proteases, alkaline proteases, cysteine proteases, or a combination thereof.

58. The method of claim 56, wherein the second enzyme mixture further comprises a second agent selected from a medium, an antibiotic, and a combination thereof.

59. The method of claim 56, wherein the medium is selected from Tyrode's solution, lactated Ringer's Solution, acetated Ringer's solution, TRIS-buffered saline (TBS), Hank's balanced salt solution (HBSS), Earle's balanced salt solution (EBSS), Standard saline citrate (SSC), HEPES-buffered saline (HBS), Gey's balanced salt solution (GBSS), minimum essential medium Eagle alpha modification (α-MEM), phosphate buffered saline (PBS), or a combination thereof.

60. The method of claim 56, wherein the antibiotic is selected from streptomycin, gentamicin, fungizone, penicillinG, amphotericin B, or a combination thereof.

61. The method of claim 56, further comprising incubating the umbilical cord blood vessel with the second enzyme mixture at a temperature ranging from about 15 °C to about 38 °C.

62. The method of claim 61, wherein the umbilical cord blood vessel is incubated with the second enzyme mixture for about 15 to about 60 minutes.

63. The method of claim 61, further comprising inactivating the enzyme.

64. The method of claim 56, further comprising adding a second medium to the umbilical cord blood vessel after incubating the umbilical cord blood vessel with the second enzyme mixture.

65. The method of claim 64, wherein the second medium is selected from Tyrode's solution, lactated Ringer's solution, acetated Ringer's solution, TRIS-buffered saline (TBS), Hank's balanced salt solution (HBSS), Earle's balanced salt solution (EBSS), Standard saline citrate (SSC), HEPES-buffered saline (HBS), Gey's balanced salt solution (GBSS), minimum essential medium Eagle alpha modification (α-MEM), phosphate buffered saline (PBS), or a combination thereof.

66. The method of claim 64, further comprising collecting a second wash eluent after adding the medium.

67. The method of claim 66, wherein the second wash eluent comprises a cell suspension of cells selected from endothelial, subendothelial cells and combinations thereof.

68. The method of claim 66, further comprising washing the second eluent with a medium.

69. The method of claim 68, wherein, the medium may be selected from Tyrode's solution, lactated Ringer's solution, acetated Ringer's solution, TRIS-buffered saline (TBS), Hank's balanced salt solution (HBSS), Earle's balanced salt solution (EBSS), Standard saline citrate (SSC), HEPES-buffered saline (HBS), Gey's balanced salt solution (GBSS), minimum essential medium Eagle alpha modification (α-MEM), phosphate buffered saline (PBS), or a combination thereof.

70. The method of claim 67, wherein the cell suspension may comprise PVMCs.

71. The method of claim 67, further comprising culturing the cell suspension in a cell culture medium.

72. The method of claim 71, wherein the cell suspension may comprise PVMCs and wherein the PVMCs are capable of adhering to a cell culture dish.

73. The method of claim 70, further comprising concentrating the PVMCs to yield a population of concentrated disaggregated PVMCs.

74. The method of claim 67, further comprising isolating the PVMCs from the cell suspension cultured in a cell culture medium.

What is claimed:

1. An isolated PVMC, capable of expressing CD146, CD105, CD166, CD44, CD73, CD90; wherein the PVMC is CD45-; and wherein the PVMC is derived from bone.

2. The isolated PVMC of claim 1, wherein the bone comprises a bone chip, a trabecular bone cavity, bone marrow, bone cavity lavage, or a combination thereof.

3. A method for making an enhanced, autologous bone graft comprising:

a. extracting a cell suspension from a first portion of bone tissue from a subject with an enzyme, mechanical force, or a combination thereof;

b. concentrating the cells in the cell suspension by buoyant density sedimentation, filtration or centrifugation to obtain a population of concentrated bone-derived PVMCs capable of expressing CD146, CD105, CD166, CD44, CD73, CD90; wherein the PVMC is CD45-; and wherein the PVMC is derived from bone; and

c. supplementing a second portion of bone tissue to be used as a bone graft from the subject with the population of concentrated bone-derived PVMCs, so as to make the enhanced, autologous bone graft.

4. The method of claim 3, wherein the first portion of bone tissue originates from the proximal region of a femur, the distal region of a femur, or a combination thereof.

5. The method of claim 3, wherein the second portion of bone tissue originates from human bones comprising at least one of an ilium crest, a femur, a patella, a tibia, a humerus, a clavicle, a rib, a scapula, or a combination thereof.

6. The method of claim 3, further comprising, supplementing the enhanced, autologous bone graft with fresh autologous bone marrow, processed autologous bone marrow, frozen autologous bone marrow, fresh autologous bone, processed autologous bone, frozen autologous bone, or a combination thereof.

7. A composition comprising a plurality of PVMCs capable of expressing CD146, CD105, CD166, CD44, CD73, CD90; wherein the PVMC is CD45-; and wherein the PVMC is derived from bone; bone marrow cells; and an acceptable carrier.

8. The composition of claim 7, wherein the plurality of PVMCs comprises PVMCs derived from bone, an umbilical cord blood vessel, or a combination thereof.

9. The composition of claim 8, wherein the bone comprises bone chips, bone marrow tissue and other tissue, compact bone, bone marrow from an intermedullary canal, a bone chip, a trabecular bone cavity, a bone cavity lavage, or combinations thereof.

10. The composition of claim 7, further comprising a scaffold material.

11. The composition of claim 10, wherein the scaffold material comprises bone chips, ceramic-based bone graft substitutes, calcium phosphate ceramics, calcium sulfate ceramics, bioglass, polymer-based bone graft substitutes, degradable and nondegradable polymers, processed allograft bone material, mineralized processed allograft, demineralized processed allograft, collagen sponges, or combinations thereof.

12. A method for isolating PVMCs from bone the method comprising: (i) providing a sample of bone tissue from a subject; (ii) extracting the PVMCs from the bone; and (iii) concentrating the extracted PVMCs.

13. The method claim 12, further comprising grinding the bone tissue.

14. The method of claim 12, wherein extracting the PVMCs comprises: (i) extracting a cell suspension from the bone tissue by enzymatic digestion, mechanical force, or a combination thereof; and (ii) separating a population of PVMCs from the cell suspension by buoyant density sedimentation, filtration, centrifugation, or a combination thereof.

15. The method of claim 14, wherein the enzymatic digestion uses one or more enzymes that cleave the attachment of a PVMC from a basement membrane of a small blood vessel.

16. The method of claim 12, wherein concentrating the extracted PVMCs is achieved by methods comprising the use of magnetic beads containing antibodies with affinity to cell surface antigens on the PVMC.

17. The method of claim 16, wherein the antibodies are selected from anti-CD146, anti-CD105, anti-CD166, anti-CD271, or a combination thereof.

18. The method of claim 14, wherein enzymatic digestion may be achieved by using one or more enzymes selected from collagenase, neutral or acidic proteases, GAGases, or metalloproteases, clostripain, serine proteases, alkaline proteases, cysteine proteases, or combinations thereof.

19. The method of claim 3, further comprising the addition of mineralized processed allograft, minimally demineralized processed allograft, partially demineralized processed allograft, demineralized processed allograft, or a combination thereof.

20. A composition comprising pure, substantially pure, or impure isolated PVMCs, wherein the PVMCs have medicinal capabilities; and wherein the pure, substantially pure or impure isolated PVMCs further comprise bone chips, fully demineralized bone chips, or partially demineralized bone chips.

21. The composition of claim 18, wherein the isolated PVMCs may be capable of expressing CD146, CD105, CD166, CD44, CD73, CD90, or a combination thereof.

22. The composition of claim 18, wherein the isolated PVMCs are isolated from bone.

23. The composition of claim 18, wherein the medicinal capabilities of the isolated PVMC are defined by the spectrum of molecules secreted by the PVMC.