METHODS OF PREPARING PERSONALIZED BLOOD VESSELS

[01] CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/714,200, filed Aug. 3, 2018.

FIELD OF DISCLOSURE

[02] The present disclosure relates to personalized blood vessels and methods of preparing personalized blood vessels. In various embodiments the personalized blood vessels produced by the methods contemplated herein may be useful in transplantation with improved host compatibility, tissue integration and reduced susceptibility to thrombosis.

BACKGROUND

[03] Vascular diseases constitute a serious and constantly growing health issue on a global scale. The options for replacement of damaged blood vessels are limited today. Autologous transplantation requires suitable vessel in the patient and leads to donor site morbidity. Synthetic vessel replacements are available, but these lack biological integration in the host tissue and long-term patency. There is a need for fully-biological patient-individualized grafts.

[04] International publication number WO/2013/136184 discloses “methods for recellurization of blood vessels;” for example “for producing an allogeneic vein, wherein a donor vein is decellularized and then recellularized using whole blood or bone marrow stem cells.”

[05] International publication number WO/2015/181245 discloses “methods for recellularization of valves in valve-bearing veins;” for example “for producing an allogeneic venous valve, wherein a donor valve-bearing vein is decellularized and then recellularized using whole blood or bone marrow stem cells.”

SUMMARY OF THE DISCLOSURE

[05a] One aspect of the present disclosure relates to method of preparing a personalized blood vessel, comprising contacting a surface of an acellular tubular scaffold with a suspension comprising a whole blood sample from a subject in need of the personalized blood vessel, wherein the whole blood sample is diluted in a physiological solution and wherein the contacting is performed for 3 to 14 days.

[06] One example of the present disclosure relates to a method of preparing a personalized blood vessel comprising, contacting a surface of an acellular tubular scaffold with an undiluted whole

blood sample from a subject in need of the personalized blood vessel, wherein the contacting is performed for more than 2 days.

[07] Another example of the present disclosure relates to a method of preparing a personalized blood vessel, comprising contacting a surface of an acellular tubular scaffold with a suspension comprising a whole blood sample from a subject in need of the personalize blood vessel, wherein the whole blood sample is diluted in a physiological solution. In some examples, the physiological solution maintains colloid oncotic pressure of the extracellular environment, buffers the pH in a CO_2 independent mantter, and/or provides a cell protective or antioxidant effect.

[08] In some examples, a population of cells present in the whole blood sample populates the scaffold.

[09] In some examples, the whole blood sample comprises one or more non-cellular factors, wherein one or more non-cellular factors of the whole blood populate the scaffold, and wherein the one or more non-cellular factors promote cellularization of the acellular tubular scaffold and host compatibility of the vessel upon grafting.

[010] In some examples, the undiluted whole blood sample or the suspension comprising the whole blood sample further comprises an anti-thrombotic factor.

[011] In some examples, the anti-thrombotic factor comprises an anticoagulant agent.

[012] In some examples, the anticoagulant agent comprises heparin or a dextran.

[013] In some examples, the heparin is present in the undiluted whole blood sample or the suspension comprising the whole blood sample at a concentration from about 0.5 IU/mL to about 150 IU/mL at the beginning of contacting the surface of the acellular tubular scaffold.

[014] In some examples, the heparin is present in the undiluted whole blood sample or the suspension comprising the whole blood sample at a concentration of about 6.7 IU/mL at the beginning of contacting the surface of the acellular tubular scaffold.

[015] In some examples, the dextran is dextran-40.

[016] In some examples, the dextran is present in the undiluted whole blood sample or the suspension comprising the whole blood sample at a concentration from about 1 g/L to about 55 g/L at the beginning of contacting the surface of the acellular tubular scaffold.

[017] In some examples, the anti-thrombotic agent comprises ascorbic acid.

[018] In some examples, the ascorbic acid is present in the undiluted whole blood sample or the suspension comprising the whole blood sample at a concentration from about 0.2 µg/mL to about 200 µg/mL at the beginning of contacting the surface of the acellular tubular scaffold.

[019] In some examples, the concentration of ascorbic acid is present in the undiluted whole blood sample or the suspension comprising the whole blood sample at a concentration of about 5 µg/mL at the beginning of contacting the surface of the acellular tubular scaffold.

[020] In some examples, the anti-thrombotic factor comprises acetylsalicylic acid.

[021] In some examples, the acetylsalicylic acid is present in the undiluted whole blood sample or the suspension comprising the whole blood sample at a concentration of from about 0.2 µg/mL to about 200 µg/mL at the beginning of contacting the surface of the acellular tubular scaffold.

[022] In some examples, the acetylsalicylic acid is present in the undiluted whole blood sample or the suspension comprising the whole blood sample at a concentration of about 5 µg/mL at the beginning of contacting the surface of the acellular tubular scaffold.

[023] In some examples, the whole blood sample or the suspension comprising the whole blood sample further comprises equal to or more than the population average physiological level of a growth factor selected from the group consisting of: granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin (IL)-3, IL-4, neutrophin (NT)-6, pleiotrophin (HB-GAM), midkine (MK), interferon inducible protein-10 (IP-10), platelet factor (PF)-4, monocyte chemotactic protein-1 (MCP-1), RANTES (CCL-5, chemokine (C-C motif) ligand 5), IL-8, IGFs, fibroblast growth factor (FGF)-l, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, transforming growth factor (TGF)-β, VEGF, platelet-derived growth factor (PDGF)-A, PDGF-B, HB-EGF, hepatocyte growth factor (HGF), tumor necrosis factor (TNF)-α, insulin-like growth factor (IGF)-1, and any combination(s) thereof.

[024] In some examples, the growth factor is a fibroblast growth factor (FGF)-2.

[025] In some examples, the FGF-2 is human FGF-2.

[026] In some examples, the FGF-2 is present in the undiluted whole blood sample or the suspension comprising the whole blood sample at a concentration from about 0.5 ng/mL to about 200 ng/mL at the beginning of contacting the surface of the acellular tubular scaffold.

[027] In some examples, the concentration of the FGF-2 is present in the undiluted whole blood sample or the suspension comprising the whole blood sample at a concentration of about 10 ng/mL at the beginning of contacting the surface of the acellular tubular scaffold.

[028] In some examples, the growth factor is a vascular endothelial growth factor (VEGF).

[029] In some examples, the VEGF is human VEGF.

[030] In some examples, the VEGF is present in the undiluted whole blood sample or the suspension comprising the whole blood sample at a concentration from about 4 ng/mL to about 800 ng/mL at the beginning of contacting the surface of the acellular tubular scaffold.

[031] In some examples, the VEGF is present in the undiluted whole blood sample or the suspension comprising the whole blood sample at a concentration of about 80 ng/mL at the beginning of contacting the surface of the acellular tubular scaffold.

[032] In some examples, the whole blood sample or the suspension comprising the whole blood sample further comprises human serum albumin, wherein the concentration of human serum albumin at the beginning of contacting the surface of the acellular tubular scaffold is about 55 g/L to about 105 g/L.

[033] In some examples, the physiological solution comprises an inorganic salt, and a buffer system.

[034] In some examples, the buffer system comprises a CO2-independent buffer system

[035] In some examples, the CO2-independent buffer system comprises a phosphate buffer system.

[036] In some examples, the physiological solution exhibits an osmotic pressure substantially similar to whole blood, which preferably is from about 270 mOsm/kg H_2O to about 310 mOsm/kg H_2O.

[037] In some examples, the physiological solution further comprises an oncotic factor and/or a nutrient.

[038] In some examples, the oncotic factor comprises serum albumin.

[039] In some examples, the concentration of human serum albumin VEGF is present in the undiluted whole blood sample or the suspension comprising the whole blood sample at a concentration from about 55 g/L to about 105 g/L at the beginning of contacting the surface of the acellular tubular scaffold.

[040] In some examples, the nutrient comprises one or more of a sugar, an amino acid, or a vitamin.

[041] In some examples, the sugar is D-glucose.

[042] In some examples, the physiological solution comprises a growth factor, and antithrombotic factor, a nutrient, and an oncotic factor; wherein the growth factor comprises human FGF-2 and human VEGF; wherein the anti-thrombotic factor comprises one or more of acetylsalicylic acid, heparin or dextran; and wherein the nutrient comprises D-glucose.

[043] In some examples, the method does not require contacting the acellular tubular scaffold with a cell culture medium after contacting with whole blood for preparing the personalized blood vessel.

[044] In some examples, contacting the surface of the acellular tubular scaffold is for more than 3 days, more than 4 days, more than 5 days, more than 6 days, more than 7 days, for 2 to 21 days, for 3 to 21 days, for 4 to 21 days, for 5 to 21 days, for 6 to 21 days, for 7 to 21 days, or for 7-9 days.

[045] In some examples, wherein the acellular tubular scaffold is contacted with the suspension comprising the whole blood sample, and the method further comprises monitoring a plurality of environmental parameters and/or a concentration of a nutrient during the preparation of the personalized blood vessel.

[046] In some examples, the methods of the present disclosure further comprise adjusting the plurality of environmental parameters.

[047] In some examples, the plurality of environmental parameters comprises temperature, pH, oxygen, and/or CO_2.

[048] In some examples, the acellular tubular scaffold is contacted with the suspension comprising the whole blood sample, and the method further comprises monitoring a concentration of a nutrient in the suspension.

[049] In some examples, the methods of the present disclosure further comprise continuously or regularly adjusting the concentration of the nutrient.

[050] In some examples, the nutrient is D-glucose, and wherein the D-Glucose concentration is adjusted continuously or regularly to maintain the D-glucose concentration at about 3 to about 11 mmol/L.

[051] In some examples, the concentration of D-glucose in the suspension is monitored by measuring the concentration of D-glucose in a sample collected from the suspension.

[052] In some examples, the concentration of D-glucose in the suspension is measured once every day.

[053] In some examples, the concentration of D-glucose in the suspension is monitored by measuring the concentration of D-glucose using a sensor that is in contact with the suspension.

[054] In some examples, the sensor is continuously in contact with the suspension during the contacting.

[055] In some examples, D-glucose is added when the measured concentration of D-glucose in the suspension is below 4 mmol/L.

[056] In some examples, D-glucose is added to reach a final concentration of 10 mmol/L of D-glucose in the suspension.

[057] In some examples, the surface of the acellular tubular scaffold is the inner surface of the acellular tubular scaffold.

[058] In some examples, the whole blood comprises peripheral blood or umbilical cord blood.

[059] In some examples, the method further comprises monitoring a concentration of human serum albumin, wherein the concentration of human serum albumin is about 55 g/L to about 105 g/L at the beginning of contacting the surface of the acellular tubular scaffold.

[060] In some examples, wherein contacting the acellular tubular scaffold results in proliferation and/or differentiation of a plurality of progenitor cells to a plurality of endothelial cells.

[061] In some examples, the plurality endothelial cells express VE-cadherin, AcLDL, vWF, and/or CD31.

[062] In some examples, the acellular tubular scaffold is a decellularized blood vessel.

[063] In some examples, the acellular tubular scaffold is continuously perfused with the suspension or whole blood.

[064] In some examples, the acellular tubular scaffold is perfused at a speed of about 0.1 mL to about 50 mL per minute.

[065] In some examples, the acellular tubular scaffold is perfused at a speed of about 2 mL per minute.

[066] In some examples, the scaffold is perfused with a closed recirculation of the suspension or whole blood.

[067] In some examples, the continuous contacting is perfusion conducted using a bioreactor.

[068] In some examples, the contacting is conducted *in vitro*.

[069] In some examples, the contacting is conducted at about 8 ºC to about 40 ºC.

[070] In some examples, wherein the contacting is conducted at about 20 ºC to about 25 ºC.

[071] In some examples, the personalized blood vessel is a vein.

[072] In some examples, the vein is a femoral vein.

[073] In some examples, the methods of the present disclosure further comprise assessing the venous valve function of the personalized blood vessel using a valve competence test.

[074] Another example of the present disclosure relates to a personalized blood vessel prepared by the method of any one of the methods of preparing a personalized blood vessel disclosed herein.

[075] In some examples, the personalized blood vessel has been implanted into a subject by surgery.

[076] Another example of the present disclosure relates to a method of surgery comprising implanting the personalized blood vessel disclosed herein into a subject in need thereof.

[077] Another example of the present disclosure relates to a personalized blood vessel for use in a method of surgery, wherein the method comprises implanting the personalized blood vessel of disclosed herein into a subject in need thereof.

[078] In some examples, the subject is human.

[079] Another example of the present disclosure relates to a bioreactor for preparing a personalized blood vessel, the bioreactor comprising a peristaltic pump, a container comprising a sampling port, a first connector, and a second connector, wherein the first and second connectors are directly or indirectly connected to the container, wherein each connector is connected to an end of a tubular scaffold for preparing a personalized blood vessel prepared by the methods disclosed herein, wherein when the first and second connectors are connected to the two ends of a tubular scaffold, the peristaltic pump mediates the circulation of a suspension or solution in a closed circuit.

[080] In some examples, the first and second connectors are Luer connectors.

[081] In some examples, the sampling port is an injection port.

[082] In some examples, the bioreactor further comprises an injection port.

[083] In some examples, the injection port is connected to a reservoir of D-glucose.

[084] In some examples, the first container is directly connected to the container by a tube, and/or the second container is directly connected to the container by a tube.

[085] In some examples, the bioreactor comprises one or more sample ports.

[086] In some examples, the bioreactor further comprises one or more sensors for measuring glucose level.

[087] In some examples, the bioreactor further comprises a pH adjusting module.

[088] In some examples, the bioreactor further comprises a CO_2 adjusting module.

[089] Another example of the present disclosure relates to a method of preparing a personalized blood vessel, the method comprising contacting a surface of an acellular tubular scaffold with a component contained in the whole blood, which is enriched or selected prior to use in contacting the surface.

[090] In some examples, the component is selected from thrombocytes, nucleated cells, proteins, growth factors, signaling factors, immunoglobulins, and any combinations thereof.

[091] In some examples, the component is enriched by centrifugation, gradient centrifugation; or selected by selective adhesion, filtration, or sorting.

[092] In some examples, the surface is an inner surface of the acellular tubular scaffold.

BRIEF DESCRIPTION OF THE DRAWINGS

[093] FIGs. 1A-1B are angiograms of a sham operated *vena cava* (FIG. 1A) and a transplanted P-TEV (FIG. 1B). Metal clips are indicated by arrow heads to localize the anastomoses.

[094] FIGs. 2A-2B are a series of images showing Hematoxylin and Eosin (H&E) staining and 4′,6-diamidino-2-phenylindole (DAPI) staining of native *vena cava* ("Native," FIG. 2A) and P-

TEV transplants ("P-TEV," FIG. 2B) from adjacent sections 3 days, 2 weeks, 17 days, and 4-5 weeks post-surgery.

[095] FIGs. 3A-3D depict images showing vena cava cellularization two weeks post surgery. FIGs. 3A-3D are a series of immunohistograms showing DAPI staining and CD31 immunostaining of *vena cava* two weeks post-surgery. FIG. 3A shows native *vena cava* proximal to the anastomoses. FIGs. 3B-3D show proximal (FIG. 3B), center (FIG. 3C), and distal (FIG. 3D) parts of the P-TEV. Arrow heads indicate CD31-positive cells.

[096] FIGs. 4A-4D depict images showing Vena cava cellularization four weeks post surgery. FIGs. 4A-4D are a series of immunohistograms showing DAPI staining and CD31 immunostaining of *vena cava* 4-5 weeks post-surgery. FIG. 4A shows native *vena cava* proximal to the anastomoses. FIGs. 4B-4D show proximal (FIG. 4B), center (FIG. 4C), and distal (FIG. 4D) parts of the P-TEV. Arrow heads indicate CD31-positive cells.

[097] FIGs. 5A-5B depict images showing luminal cell morphology four weeks post surgery. FIGs. 5A-5B is a series of images at 40× magnification showing H&E staining of native *vena cava* (FIG.

WHAT IS CLAIMED IS:

1. A method of preparing a personalized blood vessel,
comprising contacting a surface of an acellular tubular scaffold with a suspension comprising a whole blood sample from a subject in need of the personalized blood vessel, wherein the whole blood sample is diluted in a physiological solution and wherein the contacting is performed for 3 to 14 days.

2. The method of claim 1, wherein a population of cells in the whole blood sample populate the acellular tubular scaffold.

3. The method of claim 1, wherein the whole blood sample comprises one or more non-cellular factors, wherein the one or more non-cellular factors of the whole blood populate the scaffold, and wherein the non-cellular factors promote cellularization of the acellular tubular scaffold and host compatibility of the vessel upon grafting.

4. The method of claim 1, wherein the suspension comprising the whole blood sample further comprises an anti-thrombotic factor.

5. The method of claim 4, wherein the anti-thrombotic factor comprises an anticoagulant agent.

6. The method of claim 5, wherein the anticoagulant agent comprises a heparin or a dextran.

7. The method of claim 6, wherein the heparin is present in the suspension comprising the whole blood sample at a concentration from about 0.5 IU/mL to about 150 IU/mL at the beginning of contacting the surface of the acellular tubular scaffold.

8. The method of claim 7, wherein the heparin is present in the suspension comprising the whole blood sample at a concentration of about 6.7 IU/mL at the beginning of contacting the surface of the acellular tubular scaffold.

9. The method of claim 6, wherein the dextran is dextran-40.

10. The method of claim 6, wherein the dextran is present in the undiluted whole blood sample or the suspension comprising the whole blood sample at a concentration from about 1 g/L to about 55 g/L at the beginning of contacting the surface of the acellular tubular scaffold.

11. The method of claim 4, wherein the anti-thrombotic agent comprises ascorbic acid.

12. The method of claim 11, wherein the ascorbic acid is present in the suspension comprising the whole blood sample at a concentration from about 0.2 µg/mL to about 200 µg/mL at the beginning of contacting the surface of the acellular tubular scaffold.

13. The method of claim 11, wherein the ascorbic acid is present in the suspension comprising the whole blood sample at a concentration of about 5 µg/mL at the beginning of contacting the surface of the acellular tubular scaffold.

14. The method of claim 4, wherein the anti-thrombotic factor comprises acetylsalicylic acid.

15. The method of claim 14, wherein the acetylsalicylic acid is present in the suspension comprising the whole blood sample at a concentration of from about 0.2 µg/mL to about 200 µg/mL at the beginning of contacting the surface of the acellular tubular scaffold.

17. The method of claim 14, wherein the acetylsalicylic acid is present in the suspension comprising the whole blood sample at a concentration of about 5 μg/mL at the beginning of contacting the surface of the acellular tubular scaffold.

18. The method of claim 1, wherein the suspension comprising the whole blood sample further comprises equal to or more than the population average physiological level of a growth factor selected from the group consisting of: granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin (IL)-3, IL-4, neutrophin (NT)-6, pleiotrophin (HB-GAM), midkine (MK), interferon inducible protein-10 (IP-10), platelet factor (PF)-4, monocyte chemotactic protein-1 (MCP-1), RANTES (CCL-5, chemokine (C-C motif) ligand 5), IL-8, IGFs, fibroblast growth factor (FGF)-l, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, transforming growth factor (TGF)-β, VEGF, platelet-derived growth factor (PDGF)-A, PDGF-B, HB-EGF, hepatocyte growth factor (HGF), tumor necrosis factor (TNF)-α, insulin-like growth factor (IGF)-1, and any combination(s) thereof.

19. The method of claim 17, wherein the growth factor is a fibroblast growth factor (FGF)-2.

20. The method of claim 1, wherein the contacting is for a period of 3 to 9 days.

21. The method of claim 19, wherein the contacting is for a period of 4 to 9 days.