

matrix compositions that are at least 98 % decellularized such that the extracellular matrix composition is at least 98 % without cells and/or cellular remnants. In still further exemplary aspects described herein, the term "acellular" can refer to extracellular matrix compositions that are at least 99 % decellularized such that the extracellular matrix composition is at least 99 % without cells and/or cellular remnants. Thus, as used herein, the term "acellular" can refer to extracellular matrix compositions that are decellularized at levels of 80 %, 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 %, 100 %, and any percentages falling between these values.

[0044] As used herein, the term "additive" refers to materials that can be selectively incorporated into the disclosed ECM materials to impart predetermined properties to the sterilized, acellular ECM compositions disclosed herein. Such additives can include, for example and without limitation, growth factors, cytokines, proteoglycans, glycosaminoglycans (GAGs), proteins, peptides, nucleic acids, small molecules, cells and pharmaceutical agents, such as statin drugs, corticosteroids, anti-arrhythmic drugs, nonsteroidal anti-inflammatory drugs, other anti-inflammatory compounds, nanoparticles, and metallic compounds.

[0045] As used herein, the term "contemporaneously" refers to the simultaneous and/or overlapping occurrence of events, as well as the sequential occurrence of events within about thirty minutes before or after one another. Thus, if a first event occurs, then a second event can be said to have occurred contemporaneously with the first event if it occurred concurrently with the first event or within thirty minutes before or after the first event. For example, if a first method step is performed, then a second method step performed five minutes after the first method step can be said to be performed "contemporaneously" with the first method step. Similarly, if the second method step was performed ten minutes before performance of a third method step, then the second method step can be said to be performed "contemporaneously" with the third method step.

[0046] As used herein, the term "supercritical" refers to a fluid state of a material when it is held at or above its critical temperature and critical pressure. When a material is held at or above its critical temperature and critical pressure, then it typically adopts functional properties of both a gas and a liquid and is said to function as a supercritical fluid. Thus, for example, when carbon dioxide is held at or above its critical temperature (31.1°C) and its critical pressure (7.384 MPa (1,071 psi)), it behaves as a supercritical carbon dioxide fluid and can, for example, exhibit the expansion properties of a gas while having the density of a liquid.

material to one or more sterilants and additives, as disclosed herein. In exemplary aspects, the reactor vessel can be, without limitation, a basket, a bucket, a barrel, a box, a pouch, and other known containers. In one aspect, it is contemplated that the reactor vessel can be a syringe that is filled with an ECM material.

[0075] It is contemplated that a selected primary sterilant, such as, for example and without limitation, carbon dioxide, can be introduced to the reactor vessel from a header line via a valve and a supply line. It is further contemplated that a filter, such as, for example and without limitation, a 0.5 μm filter, can be provided in the supply line to prevent escape of material from the vessel. In exemplary aspects, a pressure gauge can be provided downstream of a shut-off valve in the header line to allow the pressure to be visually monitored. A check valve can be provided in the header line upstream of the valve to prevent reverse fluid flow into the booster. In order to prevent an overpressure condition existing in the header line, a pressure relief valve can optionally be provided.

[0076] In one aspect, depressurization of the reactor vessel can be accomplished using an outlet line and a valve in communication with the reactor vessel. In this aspect, it is contemplated that the depressurized fluid can exit the vessel via the supply line, be filtered by a filter unit, and then be directed to a separator, where filtered fluid, such as carbon dioxide, can be exhausted via an exhaust line. It is further contemplated that valves can be incorporated into the various lines of the apparatus to permit fluid isolation of upstream components.

[0077] In one exemplary aspect, the reactor vessel can comprise stainless steel, such as, for example and without limitation, 316 gauge stainless steel. In another exemplary aspect, the reactor vessel can have a total internal volume sufficient to accommodate the materials being sterilized, either on a laboratory or commercial scale. For example, it is contemplated that the reactor vessel can have a length of about 20 cm (8 inches), an inner diameter of about 6.4 cm (2.5 inches), and an internal volume of about 600 mL. In additional aspects, the reactor vessel can comprise a vibrator, a temperature control unit, and a mechanical stirring system comprising an impeller and a magnetic driver. In one optional aspect, it is contemplated that the reactor vessel can contain a basket comprising 316 gauge stainless steel. In this aspect, it is contemplated that the basket can be configured to hold materials to be sterilized while also protecting the impeller and directing the primary sterilant in a predetermined manner.

further, it is contemplated that the rapid depressurization of the ECM material can be used to improve retention of the tensile strength of the ECM material, as compared to previously known decellularization methods.

[0083] The disclosed methods not only do not significantly weaken the mechanical strength and bioptric properties of the ECM compositions, but also the methods are more effective in decellularizing the ECM compositions and in enhancing the incorporation of various additives into the ECM compositions. Thus, the disclosed sterilization and decellularization methods provide ECM compositions that are more decellularized and have a greater capacity to incorporate and then deliver more additives than ECM compositions known in the art. Moreover, the disclosed sterilization and decellularization methods provide ECM compositions that have greater amounts and/or concentrations of retained native growth factors and that have greater tensile strength than sterilized and decellularized ECM compositions known in the art.

[0084] In exemplary aspects, the primary sterilant can be carbon dioxide at or near its supercritical pressure and temperature conditions. However, it is contemplated that any conventional sterilant, including, for example, gas, liquid, or powder sterilants that will not interfere with the native properties of the ECM material can be used as the primary sterilant.

[0085] In one exemplary aspect, the disclosed sterilization process can be practiced using carbon dioxide as a primary sterilant at pressures ranging from about 6.9 MPa (1,000 psi) to about 24 MPa (3,500 psi) and at temperatures ranging from about 25°C to about 60°C. More preferably, when supercritical carbon dioxide is used, it is contemplated that the sterilization process can use carbon dioxide as a primary sterilant at pressures at or above 7.384 MPa (1,071 psi) and at temperatures at or above 31.1°C. In this aspect, the ECM material to be sterilized can be subjected to carbon dioxide at or near such pressure and temperature conditions for times ranging from about 10 minutes to about 24 hours, more preferably from about 15 minutes to about 18 hours, and most preferably, from about 20 minutes to about 12 hours. Preferably, the carbon dioxide employed in the disclosed systems and methods can be pure or, alternatively, contain only trace amounts of other gases that do not impair the sterilization properties of the carbon dioxide. For ease of further discussion below, the term "supercritical carbon dioxide" will be used, but it will be understood that such a term is non-limiting in that carbon dioxide within the pressure and temperature ranges as noted above can be employed satisfactorily in the practice of the disclosed methods. Within the disclosed pressure and

[00137] Within 48 hours of storage, the extracellular matrix materials were processed in supercritical carbon dioxide as disclosed herein for 20-60 minutes at temperatures at or greater than 31.1°C and pressures at or greater than 7.384 MPa (1,071 psi). After this sterilization step, the extracellular matrix materials were rapidly depressurized at a rate of 2.7 MPa/10 sec. (391.6 psi/ 10 sec.) for a minute and 19 seconds. During this time, the pressure applied to the extracellular matrix materials rapidly decreased from 9.9 MPa to 0.69 MPa.

[00138] The extracellular matrix materials were then processed in supercritical carbon dioxide and peracetic acid (PAA) as disclosed herein for 30 minutes to 6 hours to achieve terminal sterilization. In this processing step, the pressure applied to the extracellular matrix materials was increased to 9.9 MPa. The resulting sterilized, acellular extracellular matrix materials were then packaged in Tyvek® (E.I. du Pont de Nemours & Company) pouches that were sealed within plastic pouches to prevent fluid leakage.

[00139] Table 2 summarizes the sterilization and decellularization of porcine ureter, bovine pericardium, and porcine mesothelium.

Table 2

Material	Triton X-100 Conc.	Deoxycholic Acid Conc.	TX-100/Deoxy incubation	RNAse/ DNAse incubation	Supercritical CO ₂ /PAA time
Porcine ureters	0.5%	0.5%	24 hours	2 hours	120 minutes
Bovine pericardium	0.5%	0.5%	24 hours	2 hours	180 minutes
Porcine mesothelium	0.5%	0.5%	24 hours	2 hours	120 minutes

Example Seven

[00140] The DNA content of ECM material samples was measured as an indicator of decellularization of the respective ECM material samples using various sterilization and decellularization techniques. The measured DNA content was evaluated with a pico green assay in which DNA was labeled with a fluorescent label that was detected with a spectrophotometer. The measured DNA content was normalized by the dry weight of the samples. DNA content was measured and evaluated for the following treatment groups: (1) Lyophilized, non-sterile SIS; (2) Ethylene Oxide (EtO)-sterilized SIS; (3) Lyophilized, non-sterile SIS that was sterilized through a 60 minute treatment with PAA and supercritical CO₂, as disclosed herein; (4) Lyophilized, non-sterile SIS that was sterilized through a 20 minute

What is claimed is:

1. A method of producing an ECM valve conduit for regenerating a semi-lunar valve to replace a defective semi-lunar valve within a heart of a first subject, the defective semi-lunar valve being attached at an annular region between a ventricle of the heart of the first subject and an artery of the first subject, the method comprising:

providing a sheet of acellular extracellular matrix (ECM) material;

positioning said sheet of ECM material in a folded position, said sheet having a top portion comprising a top edge of said sheet and a bottom portion comprising a bottom edge of said sheet; wherein, in said folded position, said bottom edge of said sheet is folded toward said top edge of said sheet such that said bottom edge of said sheet is spaced a first distance from said top edge of said sheet;

securing said sheet of ECM material in said folded position, thereby forming said folded ECM valve construct having an upper portion, a lower portion, a first side edge, and a second side edge, said lower portion comprising a first layer and a second layer, said first layer of said lower portion corresponding to said folded bottom portion of said sheet of said ECM material;

attaching said first layer to said second layer only at locations adjacent and along said bottom edge of said sheet, wherein said first and second layers are attached at first and second attachment points, said first attachment point being spaced from said second attachment point by a second distance, wherein a sinus-promoting portion is formed on said ECM valve construct upper portion and a commissure-promoting portion is formed on said ECM valve construct lower portion first layer;

positioning said ECM valve construct in an aligned position, in which said first side edge of said ECM valve construct is in alignment with said second side edge of said ECM valve construct; and

with said ECM valve construct in said aligned position, securing said first side edge of said ECM valve construct to said second side edge of said ECM valve construct at a securement location, thereby forming said ECM valve conduit having a lumen and a longitudinal axis, wherein said lower portion of said ECM valve construct corresponds to an inlet portion of said ECM valve conduit, said inlet portion of said ECM valve conduit defining an inlet in fluid communication with said lumen of said ECM valve conduit, wherein said first layer of said lower portion of said ECM valve

construct corresponds to an inner layer positioned within said lumen of said ECM valve conduit, wherein said upper portion of said ECM construct corresponds to an outlet portion of said ECM valve conduit, and wherein said second layer of said lower portion of said ECM valve construct cooperates with said upper portion of said ECM valve construct to define an outer wall of said ECM valve conduit, wherein said inner layer of said ECM valve conduit is continuous around its circumference except at said first and second attachment points and said securement location;

said ECM valve conduit being suitable for attachment to the annular region of said heart of said first subject and said artery of said first subject, whereby said inlet portion of said ECM valve conduit is positioned proximate said annular region,

whereby, following attachment of said ECM valve conduit to said annular region of said first subject's heart and said artery of said first subject,

(i) said commissure-promoting portion of said ECM valve conduit inner layer remodels and fuses with said upper portion of said ECM valve construct, wherein a regenerated valve leaflet of a replacement semi-lunar valve is formed in said ECM valve conduit lumen, and

(ii) said sinus-promoting portion remodels and fuses with said ECM valve conduit inner layer of said ECM valve conduit, wherein a regenerated sinus portion of said replacement semi-lunar valve is formed,

(iii) said regenerated valve leaflet and said regenerated sinus portion of said ECM valve conduit comprising remodeled cardiovascular tissue.

2. The method of claim 1, wherein said first attachment point and said second attachment point are positioned on a first common plane, said first common plane being perpendicular to said longitudinal axis of said ECM valve conduit.

3. The method of claim 2, wherein said first attachment point is spaced from said first side edge by a third distance, wherein said second attachment point is spaced from said second side edge by a fourth distance, and wherein said third distance and said fourth distance are equal to said second distance by which said first attachment point is spaced from said second attachment point.

4. The method of claim 1, wherein said first layer of said lower portion of said ECM valve construct is further attached to said second layer of said lower portion of said ECM valve construct at a third attachment point intermediate said first side edge and said first attachment point, wherein said first, second, and third attachment points

are positioned on a second common plane, said second common plane being perpendicular to said longitudinal axis of said ECM valve conduit, and wherein an additional sinus-promoting portion is formed on said ECM valve construct upper portion and an additional commissure-promoting portion is formed on said lower portion first layer,

whereby said additional sinus-promoting portion is suitable, following attachment of said ECM valve conduit to said annular region of said first subject's heart and said first subject's artery, to remodel and fuse with said inner layer of said ECM valve construct,

wherein an additional regenerated sinus portion of said replacement semi-lunar valve is formed, whereby said additional commissure-promoting portion is suitable to remodel and fuse with said upper portion of said ECM valve construct, and

wherein an additional regenerated valve leaflet is formed.

5. The method of claim 4, wherein said third attachment point is spaced from said first attachment point a fifth distance, and wherein said fifth distance equal to said second distance by which said first attachment point is spaced from said second attachment point.

6. The method of claim 4, wherein said first layer of said lower portion of said ECM valve construct is further attached to said second layer of said lower portion of said ECM valve construct at a fourth attachment point intermediate said second side edge and said second attachment point, wherein the first, second, third, and fourth attachment points are positioned on said second common plane.

7. The method of claim 6, wherein said fourth attachment point is spaced from said second attachment point a sixth distance, and wherein said sixth distance equal to said second distance by which said first attachment point is spaced from said second attachment point.

8. The method of claim 1, wherein said sheet of acellular ECM material is decellularized by introducing said ECM material into a reactor pressure vessel, introducing at least a first liquid sterilant into said reactor pressure vessel at a first controlled pressurization rate, pressurizing said reactor pressure vessel at a pressure in the range of 6.9 MPa-24 MPa (1000-3500 psi), maintaining said ECM material in contact with said first liquid sterilant for a first time period in the range of 10 min. to 24 hours, and depressurizing said reactor pressure vessel at a first depressurizing rate,

said first depressurizing rate being greater than 46 kPa/s (400 psi/min).

9. The method of claim 8, wherein said first liquid sterilant comprises supercritical carbon dioxide.

10. The method of claim 8, wherein said reactor pressure vessel is maintained at a temperature of at least 31.1° C. during said pressurization of said reactor pressure vessel.

11. The method of claim 1, wherein said ECM material comprises extracellular matrix tissue from a mammalian tissue source, said mammalian tissue source being selected from the group consisting of stomach submucosa, small intestine submucosa, large intestine tissue, bladder tissue, liver tissue, heart tissue, lung tissue, kidney tissue, pancreatic tissue, prostate tissue, fetal tissue, a placenta, a ureter, vein, artery and tissue surrounding growing bone.

12. The method of claim 11, wherein said mammalian tissue source comprises mammalian tissue from a second subject.

13. The method of claim 11, wherein said ECM material includes an additional pharmacological agent.

14. The method of claim 13, wherein said pharmacological agent comprises an anti-inflammatory.

15. The method of claim 13, wherein said pharmacological agent comprises a statin.

16. The method of claim 15, wherein said statin is selected from the group consisting of atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin and simvastatin.

17. The method of claim 13, wherein said pharmacological agent comprises a growth factor.

18. The method of claim 17, wherein said growth factor is selected from the group consisting of a platelet derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor alpha (TGF-alpha), transforming growth factor beta (TGF-beta), ~~fibroblast growth factor 2 (FGF 2)~~, basic fibroblast growth factor (bFGF), vascular epithelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), ~~platelet derived growth factor (PDGF)~~, and placental growth factor (PLGF).

19. The method of claim 13, wherein said pharmacological agent comprises an anti-arrhythmic agent.