

~~What is e~~Claimed is:

1. A method for sterilizing extracellular matrix (ECM) and rendering the ECM acellular ~~an extracellular matrix (ECM) material~~, the method comprising the steps of:
providing a reactor vessel having a sealable interior space;
providing first ECM, said first ECM having a first content of colony-forming units,
positioning ~~an~~ said first ECM material within ~~an~~ said interior space of a said reactor vessel;
sealing said interior space;
introducing a first sterilant comprising carbon dioxide into ~~the~~ said vessel interior space of the reactor vessel at a supercritical pressure in the range of 1071-3500 psi and at a temperature conditions in the range of 25° - 60° C;
maintaining said vessel pressure and temperature for a first period of time in the range of 10 minutes to 24 hours, thereby sterilizing the wherein said first ECM material has a second content of said colony-forming units, said second content of colony-forming units being less than said first content of colony-forming units; and
rapidly depressurizing the said interior space of the said reactor vessel at a depressurization rate of at least 400 in the range of 800 - 2300 psi/minute, thereby rendering the wherein said first ECM material is rendered at least 96% acellular.
2. The method of claim 1, wherein ~~the depressurization rate ranges from about 400 psi/minute to about 2,600 psi/minute~~ said first ECM comprises ECM from a mammalian tissue source, said tissue source being selected from the group consisting of stomach submucosa (SS), small intestinal submucosa (SIS), large intestinal tissue, urinary bladder submucosa (UBS), liver basement membrane (LBM), heart tissue, lung tissue, kidney tissue, pancreatic tissue, prostate tissue, mesothelial tissue, fetal tissue, and placenta tissue.
3. The method of claim 1, wherein ~~the depressurization rate ranges from about 700 psi/minute to about 1,500 psi/minute~~ prior to said step of positioning said first ECM within

said reactor vessel, said method further comprises a first ECM incubation step comprising incubating said first ECM for 24 - 48 hours in 0.5-1% Triton X-100 and 0.5 - 1% deoxycholic acid with 5 mM ethylenediaminetetraacetic acid (EDTA).

4. ~~The method of claim 13, wherein the depressurization rate ranges from about 1,000 psi/minute to about 1,200 psi/minute~~ said method further comprises a second ECM incubation step comprising incubating said first ECM for 30 minutes to 24 hours in isotonic tris buffer containing 10 - 50 µg/mL of RNAase/0.2 - 0.5 µg/mL DNAase with 5 mM EDTA.

5. The method of claim 1, further comprising introducing at least one secondary sterilant into ~~the~~ said interior space of ~~the~~ said reactor vessel with said carbon dioxide.

6. A sterilized, acellular extracellular matrix (ECM) composition produced using the method of claim 1.

7. The sterilized, acellular ECM composition of claim 6, wherein the ECM composition comprises at least one tissue, each tissue of the at least one tissue being from a respective tissue source, wherein the tissue source of each tissue of the at least one tissue is selected from the group consisting of small intestinal submucosa, stomach submucosa, large intestinal tissue, urinary bladder submucosa, liver basement membrane, pericardium, epicardium, endocardium, myocardium, lung tissue, kidney tissue, pancreatic tissue, prostate tissue, mesothelial tissue, fetal tissue, a placenta, a ureter, veins, arteries, heart valves with or without their attached vessels, tissue surrounding the roots of developing teeth, and tissue surrounding growing bone.

8. The sterilized, acellular ECM composition of claim 6, wherein the ECM composition is at least about 96% decellularized.

9. A method for sterilizing, rendering acellular, and incorporating an additive into an extracellular matrix (ECM) ~~material~~, the method comprising the steps of:
providing a reactor vessel having a sealable interior space;
providing first ECM, said first ECM having a first content of colony-forming units,
positioning ~~an~~ said first ECM ~~material~~ within ~~an~~ said interior space of a said reactor vessel;
sealing said interior space;
introducing carbon dioxide into ~~the~~ said interior space of ~~the~~ reactor vessel at a supercritical pressure in the range of 1071-3500 psi and at a temperature conditions in the range of 25° - 60° C;
maintaining said vessel pressure and temperature for a first period of time in the range of 10 minutes to 24 hours, thereby sterilizing the wherein said first ECM ~~material~~ has a second content of said colony-forming units, said second content of colony-forming units being less than said first content of colony-forming units;
rapidly depressurizing ~~the~~ said interior space of ~~the~~ said reactor vessel at a depressurization rate of at least 400 in the range of 800 - 2300 psi/minute, thereby rendering the wherein said first ECM ~~material~~ is rendered acellular; and
introducing ~~one or more additives~~ at least a first additive into ~~the~~ said interior space of the said reactor vessel, whereby at least a portion of each wherein said additive of the one or more additives is incorporated into the said sterilized and acellular ~~first~~ ECM ~~material~~.
10. The method of claim 9, wherein ~~the depressurization rate ranges from about 400 psi/minute to about 2,600 psi/minute~~ said first ECM comprises ECM from a mammalian tissue source, said tissue source being selected from the group consisting of stomach submucosa (SS), small intestinal submucosa (SIS), large intestinal tissue, urinary bladder submucosa (UBS), liver basement membrane (LBM), heart tissue, lung tissue, kidney tissue, pancreatic tissue, prostate tissue, mesothelial tissue, fetal tissue, and placenta tissue.

11. The method of claim 9, wherein ~~the depressurization rate ranges from about 700 psi/minute to about 1,500 psi/minute~~ prior to said step of positioning said first ECM within said reactor vessel, said method further comprises an ECM incubation step comprising incubating said first ECM for 24 - 48 hours in 0.5 - 1% Triton X-100 and 0.5 - 1% deoxycholic acid with 5 mM ethylenediaminetetraacetic acid (EDTA).

12. ~~The method of claim 9, wherein the depressurization rate ranges from about 1,000 psi/minute to about 1,200 psi/minute.~~

~~13.~~12. The method of claim 9, further comprising introducing at least one secondary sterilant into ~~the~~ said interior space of ~~the~~ said reactor vessel with said carbon dioxide.

~~14.~~13. The method of claim 9, wherein ~~the one or more additives~~ said first additive comprises ~~at least one~~ a growth factor selected from the group consisting of transforming growth factor- β (TGF- β), fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF), placental growth factor (PGF), connective tissue growth factor (CTGF), hepatocyte growth factor (HGF), Insulin-like growth factor (IGF), macrophage colony stimulating factor (M-CSF), platelet derived growth factor (PDGF), epidermal growth factor (EGF), and transforming growth factor- α (TGF- α).

~~15.~~14. The method of claim 9, wherein ~~the one or more additives~~ said first additive comprises ~~at least one~~ a cytokine selected from the group consisting of stem cell factor (SCF), stromal cell-derived factor-1 (SDF-1), granulocyte macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-gamma), Interleukin-3, Interleukin-4, Interleukin-10, Interleukin-13, Leukemia inhibitory factor (LIF), amphiregulin, thrombospondin 1, thrombospondin 2, thrombospondin 3, thrombospondin 4, thrombospondin 5, and angiotensin converting enzyme (ACE).

~~16-15.~~ The method of claim 9, wherein ~~the one or more additives~~ said first additive comprises ~~at least one~~ a proteoglycan selected from the group consisting of heparan sulfate proteoglycans, betaglycan, syndecan, decorin, aggrecan, biglycan, fibromodulin, keratocan, lumican, epiphykan, perlecan, agrin, testican, syndecan, glypican, serglycin, selectin, lectican, versican, neurocan, and brevican.

~~17-16.~~ The method of claim 9, wherein ~~the one or more additives~~ said first additive comprises at least one glycosaminoglycan (GAG).

~~18-17.~~ The method of claim 9, wherein ~~the one or more additives~~ said first additive comprises ~~at least one of a protein, a peptide, and a nucleic acid~~ a statin.

~~19-18.~~ The method of claim ~~9~~17, wherein ~~the one or more additives comprises at least one pharmaceutical agent~~ said statin is selected from the group consisting of cerevastatin, atorvastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin.

~~20-19.~~ The method of claim 9, wherein ~~the one or more additives comprises~~ said first additive is in the form of nanoparticles.

~~21-20.~~ The method of claim 9, wherein ~~the~~ said step of introducing ~~the one or more additives~~ said first additive into ~~the~~ said interior space of ~~the~~ said reactor vessel is performed contemporaneously with ~~the~~ said step of rapidly depressurizing ~~the~~ said interior space of ~~the~~ said reactor vessel.

~~22-21.~~ A sterilized, acellular extracellular matrix (ECM) composition produced using the method of claim 9.

~~23~~.22. The sterilized, acellular ECM composition of claim ~~22~~21, wherein the ECM composition comprises at least one tissue, each tissue of the at least one tissue being from a respective tissue source, wherein the tissue source of each tissue of the at least one tissue is selected from the group consisting of small intestinal submucosa, stomach submucosa, large intestinal tissue, urinary bladder submucosa, liver basement membrane, pericardium, epicardium, endocardium, myocardium, lung tissue, kidney tissue, pancreatic tissue, prostate tissue, mesothelial tissue, fetal tissue, a placenta, a ureter, veins, arteries, heart valves with or without their attached vessels, tissue surrounding the roots of developing teeth, and tissue surrounding growing bone.

~~24~~.23. The sterilized, acellular ECM composition of claim ~~22~~21, wherein the ECM composition is at least about 96% decellularized.