

Claims:

1. ~~A method for sterilizing extracellular matrix (ECM) and rendering the ECM acellular, 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 said first ECM within said interior space of said reactor vessel;~~
 - ~~—— sealing said interior space;~~
 - ~~—— introducing a first sterilant comprising carbon dioxide into said vessel interior space at a pressure in the range of 1071-3500 psi and at a temperature 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, wherein said first ECM 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 said interior space of said reactor vessel at a depressurization rate in the range of 800-2300 psi/minute, wherein said first ECM is rendered at least 96% acellular.~~
2. The method of claim 1, wherein 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 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 3, wherein 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 $\mu\text{g/mL}$ of RNAase/0.2 - 0.5 $\mu\text{g/mL}$ DNAase with 5 mM EDTA.

5. The method of claim 1, further comprising introducing at least one secondary sterilant into said interior space of 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), 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 said first ECM within said interior space of said reactor vessel;~~
- ~~—— sealing said interior space;~~
- ~~—— introducing carbon dioxide into said interior space at a pressure in the range of 1071–3500 psi and at a temperature 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, wherein said first ECM 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 said interior space of said reactor vessel at a depressurization rate in the range of 800–2300 psi/minute, wherein said first ECM is rendered acellular; and introducing at least a first additive into said interior space of said reactor vessel, wherein said additive is incorporated into said first ECM.~~

10. The method of claim 9, wherein 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.

22. The sterilized, acellular ECM composition of claim 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.

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

Claims:

1. A method for contemporaneously sterilizing extracellular matrix (ECM) and rendering the ECM acellular, the method comprising the steps of:
 - providing a reactor vessel having a sealable interior space;
 - providing native ECM comprising a pre-processing content of colony-forming units, a pre-processing dry weight DNA greater than 16 µg/g, a pre-processing dry weight transforming growth factor beta (TGF-b) content of at least 5.5 pg/mg, and a pre-processing dry weight basic fibroblast growth factor (bFGF) content of at least 30 pg/mg,
 - positioning said ECM within said interior space of said reactor vessel;
 - sealing said interior space;
 - introducing a first sterilant comprising carbon dioxide (CO₂) into said vessel interior space at a pressure in the range of 7.38-24.13 MPa (1071-3500 psi) and at a temperature in the range of 25° - 60° C;
 - maintaining said vessel pressure and temperature for a minimum first processing time no less than 10 minutes; and
 - rapidly depressurizing said interior space of said reactor vessel at a depressurization rate in the range of 5.52-15.86 MPa/minute (800-2300 psi/minute),
 - wherein said steps of introducing said CO₂ into said vessel interior space, maintaining said vessel pressure and temperature, and rapidly depressurizing said interior space of said reactor vessel are performed at a minimum second processing time no greater than 180 minutes, and
 - wherein said ECM is rendered at least 96% acellular and comprises a post-processing content of said colony-forming units, said post-processing content of colony-forming units being less than said pre-processing content of colony-forming units,
 - said ECM further comprising a post-processing dry weight DNA content less than 1 µg/g, a post-processing dry weight TGF-b content of at least 2.8 pg/mg, and a post-processing dry weight bFGF content of at least 19 pg/mg.
2. The method of claim 1, wherein said first ECM comprises ECM from a mammalian tissuesource, 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 prior to said step of positioning said first ECM within

9. A method for contemporaneously sterilizing extracellular matrix (ECM) and rendering the ECM acellular, the method comprising the steps of:

_____ providing a reactor vessel having a sealable interior space;

_____ providing native ECM comprising a pre-processing content of colony-forming units, a pre-processing dry weight DNA greater than 16 µg/g, a pre-processing dry weight transforming growth factor beta (TGF-b) content of at least 5.5 pg/mg, and a pre-processing dry weight basic fibroblast growth factor (bFGF) content of at least 30 pg/mg,

_____ positioning said ECM within said interior space of said reactor vessel;

_____ sealing said interior space;

_____ introducing a first sterilant comprising carbon dioxide (CO₂) into said vessel interior space at a pressure in the range of 7.38-24.13 MPa (1071-3500 psi) and at a temperature in the range of 25° - 60° C;

_____ maintaining said vessel pressure and temperature for a minimum first processing no less than 10 minutes; and

_____ rapidly depressurizing said interior space of said reactor vessel at a depressurization rate in the range of 5.52-15.86 MPa/minute (800-2300 psi/minute), and

_____ introducing at least a first additive into said interior space of said reactor vessel during said rapid depressurization step,

_____ wherein said steps of introducing said CO₂ into said vessel interior space, maintaining said vessel pressure and temperature, rapidly depressurizing said interior space of said reactor vessel and introducing at least said first additive into said reactor vessel are performed at a minimum processing time no greater than 180 minutes, and

_____ wherein said ECM is rendered at least 96% acellular and comprises a post-processing content of said colony-forming units, said post-processing content of colony-forming units being less than said pre-processing content of colony-forming units,

_____ said ECM further comprising a second dry weight DNA content of less than 1 µg/g, a second dry weight TGF-b content of at least 2.8 pg/mg, and a second dry weight bFGF content of at least 19 pg/mg, said ECM further comprising said first additive.

10. The method of claim 9, wherein 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.