

CLAIMS:

1. A method for producing a bioengineered construct having an extracellular matrix with ~~increased~~an engineered average pore size comprising:

a) seeding cells capable of synthesizing extracellular matrix components within a culture vessel;

b) culturing the cells to synthesize, secrete and organize extracellular matrix components;

c) determining the desired average pore size to be engineered;

d) lyophilizing at least the resulting extracellular matrix components, wherein lyophilization comprises freezing the extracellular matrix components to a final freezing temperature of between about -100°C to about 0°C, wherein the average pore size of the porous extracellular matrix increases as the final freezing temperature increases and subsequently drying the extracellular matrix components, thereby producing a bioengineered extracellular matrix construct having an extracellular matrix with ~~increased~~the engineered average pore size.

2. The method of claim 1, wherein the bioengineered construct average pore size increases from at least 10 microns to at least 50 microns as the final freezing temperature increases from about -40°C to about -10°C, optionally wherein a final freezing temperature of about -40°C is reached to produce average pore sizes of at least 10 microns or a final freezing temperature of about -10°C is reached to produce average pore sizes of at least 30 microns.

3. The method of any one of claims 1 or 2, wherein the extracellular matrix producing cells are derived from neonate male foreskin, dermis, tendon, lung, umbilical cords, cartilage, urethra, corneal stroma, oral mucosa, intestine, bone marrow, placenta, amnion, muscle, adipose, or bone, optionally wherein the extracellular matrix producing cells are human dermal fibroblasts or human umbilical cord perivascular cells, or wherein the extracellular matrix producing cells are transfected cells, recombinant cells, or genetically engineered cells.

4. The method of any one of claims 1 to 3, wherein the bioengineered construct comprises at least one cell type in addition to the extracellular matrix producing cell type, optionally wherein the at least one additional cell type is selected from the group consisting of fibroblasts, stromal cells, and mesenchymal stem cells, and further optionally wherein the extracellular matrix producing cells and at least one additional cell type are admixed or are present in at least two separate layers.

5. The method of any one of claims 1 to 4, wherein the cells of each cell type are seeded at a combined density of between 1×10^5 cells/cm² to 6.6×10^5 cells/cm² or wherein the cells of each cell type are seeded at a combined density of greater than 100% confluence.
6. The method of any one of claims 1 to 5, wherein the extracellular matrix is at least 60 microns thick before lyophilizing and/or wherein the cells of the bioengineered construct are devitalized or decellularized before lyophilizing.
7. The method of any one of claims 1 to 6, wherein at least two bioengineered constructs are bonded together, optionally wherein the bonding occurs through an adhesive-enhancing means or by crosslinking with a crosslinking agent.
8. The method of any one of claims 1 to 7, wherein the cells are cultured in chemically defined media that is free of undefined animal organ or tissue extracts, optionally wherein the chemically defined media comprises TGF-alpha.
9. The method of any one of claims 1 to 8, wherein the cells are cultured on a porous membrane, optionally wherein the porous membrane comprises pores that are less than 6 microns in size.
10. The method of any one of claims 1 to 9, wherein the rate of reaching the final freezing temperature is decreased to increase the uniformity of average pore sizes, optionally wherein the rate of reaching the final freezing temperature is between 0.1°C and 0.5°C per minute.
11. A bioengineered construct comprising:
 - extracellular matrix-producing cells;
 - endogenous extracellular matrix produced by the extracellular matrix-producing cells;
 - wherein the extracellular matrix-producing cells are (a) devitalized; and optionally
 - wherein (b) the bioengineered construct has pores in the range between 10 microns and 150 microns in diameter, and (c) the bioengineered construct is formed by cells cultured in chemically defined media, optionally wherein the pores are in the range between 50 microns and 100 microns or between 80 microns and 100 microns, and/or the bioengineered construct is in powdered form.

12. A bioengineered construct comprising mesenchymal stem cells grown under chemically defined media conditions that are free of undefined animal organ or tissue extracts to produce a layer of extracellular matrix, which is synthesized and assembled by the mesenchymal stem cells.

13. The bioengineered construct of claim 12, wherein the mesenchymal stem cells are derived from bone marrow, umbilical cord, placenta, amnion, muscle, adipose, bone, tendon or cartilage, or wherein the mesenchymal stem cells are umbilical cord mesenchymal stem cells, optionally:

wherein the umbilical cord mesenchymal stem cells are isolated from umbilical cord blood, umbilical vein subendothelium, or Wharton's jelly, or

wherein the umbilical cord mesenchymal stem cells are human umbilical cord perivascular cells (HUCPVC).

14. The bioengineered construct of any one of claims 12 or 13, wherein the mesenchymal stem cells are human mesenchymal stem cells, and/or wherein the mesenchymal stem cells are transfected cells, recombinant cells, or genetically engineered cells.

15. The bioengineered construct of any one of claims 12 to 14, further comprising cells that are not mesenchymal stem cells, optionally wherein the non-mesenchymal stem cells are fibroblasts derived from tissue selected from the group consisting of neonate male foreskin, dermis, tendon, lung, urethra, umbilical cord, corneal stroma, oral mucosa, and intestine, and further optionally wherein the fibroblasts are human fibroblasts.

16. The bioengineered construct of any one of claim 12 to 15, wherein the bioengineered construct:

has pores in the range between 10 microns and 150 microns in diameter, optionally wherein the pores are in the range between 50 microns and 100 microns or between 80 microns and 100 microns;

has an average F_{max} of at least 0.4 Newtons;

has an ultimate tensile strength (UTS) of at least 0.4 Megapascals; and/or

has a plastic deformation tolerance of at least 0.4 times the initial length.

17. The bioengineered construct of any one of claims 12 to 16, wherein the cells of the bioengineered construct are devitalized, or wherein the bioengineered construct is decellularized, and/or dehydrated.
18. The bioengineered construct of any one of claims 12 to 17, wherein bioengineered construct further comprises one or more of Hyaluronan, CSF-3, Vitronectin, heparin, NCAM1, CXCL1, IL-6, IL-8, VEGFA, VEGFC, PDGF β , PECAM1, CDH5, ANGPT1, MMP2, TIMP 1, and TIMP3, and/or wherein the bioengineered construct further comprises an antimicrobial agent, a pharmaceutical drug, a growth factor, a cytokine, a peptide, or a protein.
19. The method of any one of claims 1 to 10, or the bioengineered construct of any one of claims 12 to 18, wherein the extracellular matrix of the bioengineered construct is crosslinked with a crosslinking agent, optionally wherein the crosslinking agent is selected from the group consisting of: carbodiimides, genipin, transglutaminase, ribose and other sugars, nordihydroguaiaretic acid (NDGA), oxidative agents, dehydrothermal (DHT), and ultraviolet (UV) light.
20. The method of any one of claims 1 to 10 or 19 or the bioengineered construct of any one of claims 12 to 19, wherein the bioengineered construct is contracted to at least 50% decrease in surface area by releasing the bioengineered construct from the culture substrate before lyophilizing.
21. The bioengineered construct of any one of claims 12 to 20, wherein the bioengineered construct is terminally sterilized, or the method of any one of claims 1 to 10, 19 or 20, wherein the bioengineered construct is terminally sterilized after lyophilizing.
22. A multilayered bioengineered construct, wherein at least two bioengineered constructs of any one of claims 12 to 21 are bonded together, optionally wherein the bonded bioengineered constructs are crosslinked with a crosslinking agent.