

USE OF MICROPARTICLES AND ENDOTHELIAL CELLS WITH DECELLULARIZED ORGANS AND TISSUES

Cross-Reference to Related Applications

5 This application claims the benefit of the filing date of U.S. application
Serial No. 61/790,118, filed on March 15, 2013, the disclosure of which is
incorporated by reference herein.

Background

10 Tissue engineering is a rapidly growing field that seeks to repair or
regenerate damaged or diseased tissues and organs through the implantation of
combinations of cells, scaffolds and soluble mediators. Current stem cell
differentiation and primary cell culture is generally achieved under 2-
dimensional (2D) culture conditions. That system allows for the expansion of
15 specific cell populations but is limited in its ability retain functional cellular
phenotypes, to support high density cell culture and long term primary or
differentiated cell function. For example, in contrast to the limited availability
of large numbers of primary cells needed for certain cellular therapies, the
number of stem cells can be greatly expanded while retaining the ability to
20 differentiate into specific lineages. The control of stem cell fate (e.g.,
differentiation), either *in vivo* or *in vitro*, has been attributed principally to
genetic and molecular mediators (e.g., growth factors and transcription factors).
Although stem and progenitor cell differentiation can result in cells with
appropriate lineage- or tissue-specific gene expression, the differentiated cells
25 can lack functional properties needed for *in vitro* or *in vivo* applications.

Summary of the Invention

30 In one aspect, The invention provides an *ex vivo* method to maintain, reduce a
decrease in or expand capillary vessel lumen diameter in a re-endothelialized
and/or recellularized (with cells other than endothelial cells) extracellular matrix
of a mammalian organ, tissue or portion thereof with an intact vascular bed, for
example, to ensure proper capillary diameter to sustain continuous blood flow
upon transplantation. The method includes providing or preparing a
decellularized extracellular matrix of a mammalian organ, tissue or portion

WHAT IS CLAIMED IS:

1. A method to maintain, reduce a decrease in or expand capillary vessel lumen diameter in a re-endothelialized extracellular matrix of a mammalian organ, tissue or portion thereof with an intact vascular bed, comprising:
 - 5 providing a decellularized extracellular matrix of a mammalian organ, tissue or portion thereof with an intact vascular bed and a population of endothelial cells or stem or progenitor cells capable of differentiation into endothelial cells; and
 - 10 introducing an amount of the cells and a first aqueous solution comprising an amount of biocompatible microparticles to the decellularized extracellular matrix, wherein the amount of the cells is effective to re-endothelialize the vasculature of the decellularized extracellular matrix and wherein the amount of the microparticles when circulated through the
 - 15 vasculature maintains, reduces a decrease or expands capillary vessel lumen diameter in the vasculature during or after re-endothelialization relative to a corresponding re-endothelialized decellularized extracellular matrix that lacks the microparticles.
- 20 2. The method of claim 1 wherein the microparticles maintain flow through the capillary beds.
3. The method of claim 1 or 2 wherein the microparticles are biodegradable.
- 25 4. The method of claim 1 or 2 wherein the microparticles are not biodegradable.
5. The method of any one of claims 1 to 4 wherein the microparticles are spherical or elliptical.
- 30 6. The method of any one of claims 1 to 5 wherein the microparticles are deformable.

7. The method of any one of claims 1 to 6 wherein the microparticles are formed of polymers.
8. The method of claim 7 wherein the polymer is a naturally occurring
5 polymer.
9. The method of any one of claims 1 to 6 wherein the microparticles comprise alginate, polysaccharide, collagen, dextran, hyaluronic acid, glass, ceramic, metal, poly-lactic acid (PLA), poly-glutamic acid (PGA) or co-
10 polymers of PLA and PGA (PLA/PGA).
10. The method of any one of claims 1 to 6 wherein the polymer is a non-naturally occurring polymer.
11. The method of any one of claims 1 to 10 wherein the microparticles are
15 modified to include carboxylates, esters, amines, aldehydes, alcohols, or halides.
12. The method of any one of claims 1 to 11 wherein the microparticles are formed of protein and non-protein polymers.
13. The method of any one of claims 1 to 12 wherein the average diameter of
20 the microparticles is from about 0.5 μm to about 20 μm .
14. The method of any one of claims 1 to 13 wherein the microparticles comprise a hydrophilic surface.
15. The method of any one of claims 1 to 14 wherein the microparticles are magnetic.
16. The method of any one of claims 1 to 15 wherein the microparticles
25 comprise a surface modification that binds a ligand.
17. The method of claim 1 or 6 wherein the microparticles comprise a hydrogel.

18. The method of any one of claims 1 to 17 wherein the solution is added after re-endothelialization.
19. The method of any one of claims 1 to 18 further comprising introducing a second aqueous solution comprising biocompatible microparticles having an average diameter that is at least 10% greater than the microparticles in the first solution.
20. The method of any one of claims 19 further comprising introducing a third aqueous solution comprising biocompatible microparticles having an average diameter that is at least 10% greater than the microparticles in the second solution.
21. The method of any one of claims 1 to 19 wherein the first solution comprises about 300, to about 500,000 microparticles per μL .
22. The method of any one of claims 1 to 20 further comprising washing the vasculature with a solution that lacks the microparticles.
23. The method of claim 22 wherein the solution that lacks the nanoparticles or microparticles comprises an agent that degrades the microparticles.
24. The method of claim 22 wherein the solution that lacks the nanoparticles or microparticles is applied concurrent with an external factor that degrades or removes the microparticles including temperature, pH, ultrasound, light or electrical energy.
25. The method of any one of claims 1 to 7, 10 to 11, 13 to 16, or 18 to 24 wherein the microparticles comprise polystyrene.
26. The method of any one of claims 1 to 8, 10 to 11, 13 to 16, or 18 to 24 wherein the microparticles comprise polysaccharide.
27. The method of claim 25 or 26 wherein the microparticles have an average diameter of about 5 to about 20 microns.

28. The method of any one of claims 1 to 27 wherein the organ is a heart, a pancreas, a bone, a liver, a kidney, or a lung.

29. The method of any one of claims 1 to 28 wherein the cells are obtained
5 from iPS cells.

30. The method of any one of claims 1 to 29 wherein the population is introduced to the matrix either by injection or perfusion, or a combination thereof.
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31. The method of any one of claims 1 to 30 wherein the population comprises primary cells.

32. The method of any one of claims 1 to 31 wherein the population
15 comprises a plurality of different cell types.

33. The method of any one of claims 1 to 32 wherein the population comprises human embryonic stem cells.

20 34. The method of any one of claims 1 to 33 wherein the cells and the perfusion decellularized organ or tissue are allogeneic.

25 35. The method of any one of claims 1 to 33 wherein the cells and the perfusion decellularized organ or tissue are xenogeneic.

WHAT IS CLAIMED IS:

1. An *ex vivo* method to maintain, reduce a decrease in or expand capillary vessel lumen diameter in a re-endothelialized extracellular matrix of a mammalian organ, tissue or portion thereof with an intact vascular bed, comprising:
 - providing a decellularized extracellular matrix of a mammalian organ, tissue or portion thereof with an intact vascular bed and a population of endothelial cells or stem or progenitor cells capable of differentiation into endothelial cells; and
 - introducing an amount of the cells and a first aqueous solution comprising an amount of biocompatible microparticles to the decellularized extracellular matrix, wherein the amount of the cells is effective to re-endothelialize the vasculature of the decellularized extracellular matrix and wherein the amount of the microparticles when circulated through the vasculature maintains, reduces a decrease or expands capillary vessel lumen diameter in the vasculature during or after re-endothelialization relative to a corresponding re-endothelialized decellularized extracellular matrix that lacks the microparticles.
2. The method of claim 1 wherein the microparticles maintain flow through the capillary beds.
3. The method of claim 1 or 2 wherein the microparticles are biodegradable.
4. The method of claim 1 or 2 wherein the microparticles are not biodegradable.
5. The method of any one of claims 1 to 4 wherein the microparticles are spherical or elliptical or are deformable.
6. The method of any one of claims 1 to 5 wherein the microparticles are formed of polymers.

7. The method of any one of claims 1 to 5 wherein the microparticles comprise alginate, polysaccharide, collagen, dextran, hyaluronic acid, glass, ceramic, metal, poly-lactic acid (PLA), poly-glutamic acid (PGA) or co-polymers of PLA and PGA (PLA/PGA) or are magnetic.
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8. The method of any one of claims 1 to 5 wherein the microparticles are modified to include carboxylates, esters, amines, aldehydes, alcohols, or halides.
9. The method of any one of claims 1 to 5 wherein the microparticles are formed of protein and non-protein polymers.
- 10 10. The method of any one of claims 1 to 9 wherein the average diameter of the microparticles is from about 0.5 μm to about 20 μm .
11. The method of any one of claims 1 to 10 wherein the microparticles comprise a hydrophilic surface.
12. The method of any one of claims 1 to 11 wherein the microparticles
- 15 comprise a surface modification that binds a ligand.
13. The method of claim 1 or 5 wherein the microparticles comprise a hydrogel.
14. The method of any one of claims 1 to 13 wherein the solution is added after re-endothelialization.
- 20 15. The method of any one of claims 1 to 14 further comprising introducing a second aqueous solution comprising biocompatible microparticles having an average diameter that is at least 10% greater than the microparticles in the first solution and optionally further comprising introducing a third aqueous solution comprising biocompatible microparticles having an average diameter that is at
- 25 least 10% greater than the microparticles in the second solution.
16. The method of any one of claims 1 to 15 wherein the first solution comprises about 300, to about 500,000 microparticles per μL .

17. The method of any one of claims 1 to 15 further comprising washing the vasculature with a solution that lacks the microparticles.
18. The method of claim 17 wherein the solution that lacks the nanoparticles or microparticles comprises an agent that degrades the microparticles.
- 5 19. The method of claim 17 wherein the solution that lacks the nanoparticles or microparticles is applied concurrent with an external factor that degrades or removes the microparticles including temperature, pH, ultrasound, light or electrical energy.
20. The method of any one of claims 1 to 6, 8, 10 to 12, or 14 to 19 wherein
10 the microparticles comprise polystyrene or polysaccharide.
21. The method of claim 20 wherein the microparticles have an average diameter of about 5 to about 20 microns.
22. The method of any one of claims 1 to 21 wherein the organ is a heart, a pancreas, a bone, a liver, a kidney, or a lung.
- 15 23. The method of any one of claims 1 to 22 wherein the cells are obtained from iPS cells.
24. The method of any one of claims 1 to 23 wherein the population
20 comprises primary cells, human embryonic stem cells, or a plurality of different cell types.
25. The method of any one of claims 1 to 24 wherein the cells and the perfusion decellularized organ or tissue are allogeneic or xenogenic.
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