USE OF PERFUSION DECELLULARIZED LIVER FOR ISLET CELL RECELLULARIZATION

Cross-Reference to Related Applications

This application claims the benefit of the filing date of U.S. application Serial No. 61/789,927, filed on March 15, 2013, the disclosure of which is incorporated by reference herein.

Background

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-dimentional (2D) culture conditions. That system allows for the expansion of 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 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 can lack functional properties needed for *in vitro* or *in vivo* applications.

Summary of the Invention

The invention provides an ex vivo method to prepare a graft comprising a recellularized extracellular matrix of a mammalian liver, liver lobe or portion thereof. In one embodiment, the portion is a total inflated volume of greater than or equal to about 15 cm^3. The method includes selecting a perfusion decellularized extracellular matrix of a mammalian liver, a liver lobe or a portion thereof and two populations of cells. The first population of mammalian cells includes endothelial cells or stem or progenitor cells capable of differentiation

WHAT IS CLAIMED IS:

1. An ex vivo method to prepare a graft comprising a recellularized extracellular matrix of a mammalian liver, liver lobe or portion thereof, comprising:

selecting a perfusion decellularized extracellular matrix of a mammalian liver, a liver lobe or a portion thereof and two populations of cells including a first population of mammalian cells including endothelial cells or stem or progenitor cells capable of differentiation into endothelial cells, and a second population of mammalian cells including islet cells, beta cells, insulin like cells, or stem cells or progenitor cells capable of differentiation into islet cells or beta cells; and

contacting the perfusion decellularized extracellular matrix and the two population of cells under conditions and for a period of time that provide for re-endothelialization of the vasculature of the perfusion decellularized extracellular matrix and recellularization of the perfusion decellularized extracellular matrix with the islet cells, beta cells or insulin like cells, or recellularization and differentiation and functional maturation of the stem or progenitor cells into islet cells, beta cells or insulin like cells in the perfusion decellularized extracellular matrix.

2. The method of claim 1 wherein the selected perfusion decellularized extracellular matrix is a perfusion decellularized extracellular matrix of a liver.

3. The method of claim 1 wherein the selected perfusion decellularized extracellular matrix is a perfusion decellularized extracellular matrix of a liver lobe.

4. The method of claim 1 wherein the selected perfusion decellularized extracellular matrix is a portion >8 cm^3 of a perfusion decellularized extracellular matrix of a liver lobe.

5. The method of any one of claims 1 to 4 wherein the cells in the first and the second populations are xenogeneic to the decellularized extracellular matrix.

6. The method of any one of claims 1 to 4 wherein the cells in the first and the second populations are allogeneic to the decellularized extracellular matrix.

7. The method of any one of claims 1 to 6 wherein the cells in the first population, the second population, or both, include iPS cells.

8. The method of any one of claims 1 to 7 wherein the perfusion decellularized extracellular matrix contains an intact vascular network.

9. The method of any one of claims 1 to 8 wherein the conditions include perfusing the matrix with media.

10. The method of claim 9 wherein the media contain activators or inhibitors of differentiation pathways selected to provide for cell-specific differentiation including Activin A, Retinoic Acid, bFGF, clozapine-N-oxide, phosphoinositide-3-kinase inhibitor, Nicotinamide, or a combination thereof..

11. The method of any one of claims 1 to 10 wherein the first population of cells is contacted with the extracellular matrix either by injection or perfusion, or a combination thereof.

12. The method of any one of claims 1 to 10 wherein the second population of cells is contacted with the extracellular matrix either by injection or perfusion, or a combination thereof.

13. The method of any one of claims 1 to12 wherein the first population, the second population, or both, comprise primary cells.

14. The method of any one of claims 1 to 13 wherein the second population comprises a plurality of different cell types.

15. The method of any one of claims 1 to 14 wherein the first population, the second population, or both, comprise human embryonic stem cells.

16. The method of any one of claims 1 to 14 wherein the insulin producing cells are encapsulated.

17. The method of any one of claims 1 to 5 or 7 to16 wherein the extracellular matrix is from a nonhuman mammal and is populated with human cells.

18. The method of any one of claims 1 to 17 further comprising implanting the extracellular matrix in a mammal.

19. The method of claim 18 wherein the extracellular matrix is implanted in the mammal prior to contact with the first population, the second population, or both.

20. The method of claim 19 wherein the cells in the first population, the second population, or both, are injected into the mammal.

21. The method of claim 18 wherein the extracellular matrix is implanted in a mammal after contact with the first population, but before the second population.

22. The method of claim 17 wherein extracellular matrix is implanted in a mammal after contact with the second population but before contact with the first population.

23. The method of claim 22 wherein the extracellular matrix is implanted in a mammal after contact with the first population and the second population.

24. A recellularized matrix prepared by the method of any one of claims 1 to 23.

~~25. A method to enhance insulin control in a mammal that lacks or has reduced insulin control, comprising:~~

providing a re-endothelialized extracellular matrix of a mammalian liver, a liver lobe or a portion thereof having a population of mammalian cells including islet cells, beta cells, islet like cells, or stem cells or progenitor cells capable of differentiation into islet cells or beta cells, prepared by introducing mammalian endothelial cells or cells capable of differentiation into mammalian endothelial cells and the population to a decellularized extracellular matrix of the mammalian liver, liver lobe or portion thereof; and

introducing to a mammal that lacks or has reduced insulin control the re-endothelialized matrix having the population of cells including islet cells, beta cells, islet like cells or stem cells or progenitor cells capable of differentiation into islet or beta cells, so as to provide for control of blood glucose in the mammal.

26. A method to enhance insulin control in a mammal that lacks or has reduced insulin control, comprising:

providing a re-endothelialized extracellular matrix of a mammalian liver, a liver lobe or a portion thereof prepared by introducing mammalian endothelial cells or cells capable of differentiation into mammalian endothelial cells, to a decellularized extracellular matrix of mammalian liver, liver lobe or portion thereof;

introducing to a mammal that lacks or has reduced insulin control the re-endothelialized matrix; and

introducing to the mammal having the re-endothelialized matrix a population of cells including islet cells, beta cells, islet like cells or stem cells or progenitor cells capable of differentiation into islet or beta cells, in an amount effective to provide for control of blood glucose in the mammal.

27 A method to enhance insulin control in a mammal that lacks or has reduced insulin control, comprising:

providing a mammal that lacks or has reduced insulin control and has an implanted re-endothelialized matrix of a mammalian liver, a liver lobe or a portion thereof, prepared by introducing mammalian endothelial cells to a decellularized extracellular matrix of a mammalian liver, liver lobe or portion thereof; and

~~introducing to the mammal a population of cells including islet cells, beta cells, islet like cells, or stem cells or progenitor cells capable of differentiation into islet cells, in an amount effective to provide for control of blood glucose in the mammal.~~

~~28. The method of claim 25, 26 or 27 wherein the cells are intravenously introduced to the mammal.~~