ACELLULAR SUBSTRATES AND METHODS OF MAKING THE SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 63/296,274, filed January 4, 2022, which is expressly incorporated by reference herein in its entirety.

TECHNICAL FIELD

Embodiments of the presently-disclosed invention relate generally to acellular substrates that provide continuous 3D growth of a variety of cells and/or tissues and methods of making the same. The acellular substrates have a three-dimensional (3D) macrostructure defined by a continuous matrix of extracellular matrix (ECM) material associated with a first cell type of interest and a network of microporous channels and/or chambers extending throughout the continuous matrix of ECM material associated with the first cell type of interest. Methods of forming a personalized graft using a patient's own cells is also provided.

BACKGROUND

Connective tissues, such as bone, cartilage, skin, nerve, lung, muscle, and many others contain cells that secrete and organize extracellular matrix (ECM) proteins to create scaffolds to allow cells to migrate, proliferate, and organize to form tissues. The interplay between the organization of composition and structure of ECM is well documented. For instance, each tissue has a different composition and organizational structure to instruct cell types how to arrange, organize, and function.

There remains a need in the art for the development of scaffolds that enable the creation of biological scaffolds from any connective tissue of interest, such as an ECM scaffold (e.g., acellular substrate) that can accept autologous cells from a patient to build a personalized graft.

SUMMARY

One or more examples of the disclosure may address one or more of the aforementioned problems. In a first aspect, the present disclosure provides an acellular substrate (e.g., a scaffolding) comprising a three-dimensional (3D) macrostructure defined by a continuous matrix of extracellular matrix (ECM) material associated with one or more cell types of interest from a patient and a network

of microporous channels and/or chambers extending throughout the continuous matrix of ECM material.

The 3D macrostructure may comprise a top surface, a bottom surface, and a thickness defined by at least one side edge extending from the top surface to the bottom surface. The 3D macrostructure has at least one interlocking-male component or at least one interlocking-female component or both. The network of microporous channels and/or chambers comprises a scaffold custom to the patient and generated from (i) decellularizing a tissue derived from the patient after having grown the tissue around a degradable hydrogel inverse mold of microstrands and/or micropods and (ii) degrading and removing the mold. The acellular substrate consists of a block configured such that a plurality of respective acellular substrates may be interlocked or otherwise joined together in one or more shapes.

In a second aspect, the present disclosure provides a method of forming an acellular substrate, comprising: (i) forming or placing a network of microstrands and/or micropods comprising a degradable hydrogel material within a mold that is custom to a patient and has at least one interlocking-male component or at least one interlocking-female component or both; (ii) seeding the network of microstrands and/or micropods by adding an initial culture media including cells of one or more cell types of interest from the patient into the mold housing the network of microstrands and/or micropods; (iii) feeding the cells by perfusing fresh culture media through the mold to provide the cells with nutrients until a tissue has grown and expanded to fill the mold; (iv) performing a decellularization operation on the tissue located in the mold forming a continuous matrix of ECM material associated with the one or more cell type of interest; and (v) forming a network of microporous channels and/or chambers extending throughout the continuous matrix of ECM material by degrading and removing the network of microstrands and/or micropods to provide the acellular substrate, wherein the acellular substrate consists of a block configured such that a plurality of respective acellular substrates may be interlocked or otherwise joined together in one or more shapes.

In a third aspect, the present disclosure provides a method of forming a personalized graft, comprising: (i) providing or forming one or more acellular substrates according to the first aspect, wherein the one or more cell types of interest is associated with the patient's tissue having an anomaly, such as a particular organ tissue; (ii) seeding the one or more acellular substrates with healthy native cells associated with the patients tissue having an anomaly; and (iii) feeding the healthy native cells with a culture media, and allowing the healthy native cells to propagate throughout the network of microporous channels and/or chambers of the acellular substrate forming the personalized graft.

BRIEF DESCRIPTION OF THE DRAWING(S)

The invention now will be described more fully hereinafter with reference to the accompanying drawings, in which some, but not all embodiments of the invention are shown. Indeed, this invention may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. Like numbers refer to like elements throughout, and wherein:

Figure 1 illustrates an acellular substrate in accordance with certain embodiments of the invention;

THAT WHICH IS CLAIMED:

1. An acellular substrate, comprising:

a three-dimensional (3D) macrostructure defined by a continuous matrix of extracellular matrix (ECM) material associated with one or more cell types of interest from a patient and a network of microporous channels and/or chambers extending throughout the continuous matrix of ECM material, wherein:

the 3D macrostructure comprises a top surface, a bottom surface, and a thickness defined by at least one side edge extending from the top surface to the bottom surface, and the 3D macrostructure has at least one interlocking-male component or at least one interlocking-female component or both;

the network of microporous channels and/or chambers comprises a scaffold custom to the patient and generated from (i) decellularizing a tissue derived from the patient after having grown the tissue around a degradable hydrogel inverse mold of microstrands and/or micropods and (ii) degrading and removing the mold; and

the acellular substrate consists of a block configured such that a plurality of respective acellular substrates may be interlocked or otherwise joined together in one or more shapes.

2. The acellular substrate of claim 1, wherein the acellular substrate further comprises at least one interlocking-male component and at least one interlocking-female component.

3. The acellular substrate of claim 2, wherein the at least one interlocking-male component includes a first interlocking-male component extending outwardly from the at least one side edge.

4. The acellular substrate of claim 2, wherein the at least one side edge includes a first side edge and a second side edge, and wherein the at least one interlocking-male component

includes a first interlocking-male component extending outwardly from the first side edge and a second interlocking-male component extending outwardly from the second side edge.

5. The acellular substrate according to any one of claims 2-4, wherein the at least one interlocking-female component includes a first interlocking-female component extending inwardly from the at least one side edge towards an interior portion of the 3D macrostructure.

6. The acellular substrate according to any one of claims 4-5, wherein the at least one side edge includes a third side edge and a fourth side edge, and wherein the at least one interlocking-female component includes a first interlocking-female component extending inwardly from the third side edge towards an interior portion of the 3D macrostructure and a second interlocking-female component extending inwardly from the fourth side edge towards an interior portion of the 3D macrostructure.

7. The acellular substrate of claim 6, wherein the first side edge and the third side edge define a first pair of opposing side edges, and the second side edge and the fourth side edge define a second pair of opposing side edges.

8. The acellular substrate according to any one of claims 1-7, wherein (i) the top surface comprises a macroscopic surface area from 0.25 cm^2 to 25 cm^2; (ii) the bottom surface comprises a macroscopic surface area from 0.25 cm^2 to 25 cm^2; (iii) the thickness of the 3D macrostructure is from 0.5 cm to 3 cm; or (iv) any combination of (i), (ii), and (iii).

9. The acellular substrate according to any one of claims 2-8, wherein each of the at least one interlocking-female component is configured to receive a corresponding at least one interlocking-male component of a second acellular substrate.

10. The acellular substrate according to any one of claims 1-9, wherein (i) the network of microporous channels and/or chambers extending throughout the continuous matrix of ECM material has an average diameter from 100 to 800 microns; (ii) the network of microporous channels and/or chambers extending throughout the continuous matrix of ECM material comprises at least 40% by volume of the 3D macrostructure; or (iii) both (i) and (ii).

11. A method of forming an acellular substrate, comprising:

(i) forming or placing a network of microstrands and/or micropods comprising a degradable hydrogel material within a mold that is custom to a patient and has at least one interlocking-male component or at least one interlocking-female component or both;

(ii) seeding the network of microstrands and/or micropods by adding an initial culture media including cells of one or more cell types of interest from the patient into the mold housing the network of microstrands and/or micropods;

(iii) feeding the cells by perfusing fresh culture media through the mold to provide the cells with nutrients until a tissue has grown and expanded to fill the mold;

(iv) performing a decellularization operation on the tissue located in the mold forming a continuous matrix of ECM material associated with the one or more cell types of interest; and

(v) forming a network of microporous channels and/or chambers extending throughout the continuous matrix of ECM material by degrading and removing the network of microstrands and/or micropods to provide the acellular substrate, wherein the acellular substrate consists of a block configured such that a plurality of respective acellular substrates may be interlocked or otherwise joined together in one or more shapes.

12. The method of claim 11, wherein forming or placing a network of microstrands and/or micropods comprises performing at least one additive manufacturing technique, such as 3D printing and/or digital light synthesis printing.

13. The method according to any one of claims 11-12, wherein a structure of the network of microstrands and/or micropods is selected based on at least one cell morphology of interest, in which the cell morphology has a target matrix structure and a target microporous network of channels and/or chambers; wherein the structure of the network of microstrands and/or micropods mimics and/or is identical to the target microporous network of channels and/or chambers.

14. The method according to any one of claims 11-13, wherein the network of microstrands and/or micropods has an average diameter from 100 to 800 microns.

15. The method according to any one of claims 11-14, wherein the network of microstrands and/or micropods comprises a selectably degradable hydrogel material, one or more degradable polymers, one or more biopolymers, one or more polymers derived from a living organism, or any combination thereof.

16. The method according to claim 15, wherein the network of microstrands and/or micropods comprises at least one collagen, gelatin, laminin, alginate, glycosaminoglycan, oligonucleotide, carbohydrate, lipid, cellulose, and/or protein such as those that are degradable through biopolymer chain disruption and/or with the use of one or more specific enzymes, ionic solvents, neutral detergents, weak acids, and peroxides.

17. The method according to any one of claims 15-16, wherein the network of microstrands and/or micropods comprises at least one synthetic polymer, polyester, polyanhydride, polycarbonate, polyurethane, polyphosphate, or any combination thereof.

18. The method according to any one of claims 11-17, wherein performing the decellularization operation on the tissue located in the mold comprises treating the tissue with

a detergent followed by dialyzing the continuous matrix of ECM material associated with the one or more cell types of interest.

19. The method according to any one of claims 11-18, further comprising (i) a step of lyophilizing the continuous matrix of ECM material associated with the one or more cell types of interest; (ii) a step of sterilizing the acellular substrate, such as by e-beam or gamma irradiation operations; (iii) a step of removing the acellular substrate from the mold; or (iv) any combination of (i), (ii), and (iii).

20. A method of forming a personalized graft, comprising:

(i) providing or forming one or more acellular substrates according to any one of claims 1-10, wherein the one or more cell types of interest is associated with the patient's tissue having an anomaly, such as a particular organ tissue;

(ii) seeding the one or more acellular substrates with healthy native cells associated with the patient's tissue having an anomaly; and

(iii) feeding the healthy native cells with a culture media, and allowing the healthy native cells to propagate throughout the network of microporous channels and/or chambers of the acellular substrate forming the personalized graft.

21. The method of claim 20, wherein the one or more acellular substrates comprises a first acellular substrate and a second acellular substrate, wherein the first acellular substrate and the second acellular substrate are the same.

22. The method of claim 21, wherein the first acellular substrate is joined to the second acellular substrate such that a multi-acellular scaffolding is provided, and allowing the healthy native cells to propagate throughout an aggregate network of microporous channels and/or chambers of the multi-acellular scaffolding forming the personalized graft.

23. The method according to any one of claims 20-22, wherein the one or more acellular substrates comprises at least 2 acellular substrates joined together to define a scaffolding.