TECHNIQUE FOR FORMATION AND ASSEMBLY OF 3D CELLULAR STRUCTURES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore provisional application No. 10201408826X, filed 31 December 2014, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the field of tissue engineering. In particular, the present invention relates to the formation and assembly of 3D cellular structures.

BACKGROUND OF THE INVENTION

[0003] Tissue engineering aims to create functional cellular constructs to replace damaged tissues or organs. To create tissue constructs, cells are grown on scaffolds or in gels that contain biochemical and physical cues to organize cells three dimensionally (3D). As a result, tissue constructs created using existing 3D cellular organization techniques often contain scaffold or gel materials. However, before these 3D tissue constructs can be implanted, issues such as the material biocompatibility (before and after degradation), ability of the material to support vascularization and stability of the 3D structure after material degradation need to be addressed.

[0004] There is therefore a need to provide alternative constructs and methods for producing 3D tissue constructs obviating one or more of the above disadvantages.

SUMMARY

[0005] In one aspect, the present invention refers to a method of making a self-supporting cellular construct having a continuous channel within its central cavity, wherein the method comprises the steps of: a. providing a mould with a central opening, wherein the mould encloses a volume around the central opening (hole) capable of housing a plurality of cells; b. inserting a plurality of cells in a cell culture medium into the mould; c. allowing the cells to grow and form a self-supporting cellular construct within the mould; d. removing the self-

supporting cellular construct having a central opening and an outer dimension of the mould from the mould; e. repeating a to d to obtain a plurality of self-supporting cellular construct having a central opening; f. placing each of the plurality of self-supporting cellular constructs having a central opening in a series adjacent to one another such that the central opening of each of the self-supporting cellular constructs having a central opening is aligned to one another to thereby form a continuous channel within its central cavity, wherein the plurality of cells comprises at least two cell types, wherein one cell type is capable of forming cell aggregates and the other cell type is not capable of forming cell aggregates by itself. In another aspect, the present invention refers to a method of making a self-supporting cellular construct, the method comprises the steps of: providing a mould of a desired shape, wherein the mould comprises an area capable of housing a plurality of cells and a removable material within the mould; inserting a plurality of cells in a cell culture medium into the mould; inserting a removable material before or after (b); allowing the cells to grow and form a self-supporting cellular structure surrounding the removable material within the mould; and removing the self-supporting cellular structure from the mould to thereby form a self-supporting cellular construct wherein the plurality of cells comprises at least two cell types, wherein one cell type is capable of forming cell aggregates and the other cell type is not capable of forming cell aggregates by itself. Also described herein is a method of making a self-supporting cellular construct having a continuous channel within its central cavity, wherein the method comprises the steps of providing a mould with a central opening, wherein the mould encloses a volume around the central opening (hole) capable of housing a plurality of cells; inserting a plurality of cells in a cell culture medium into the mould; allowing the cells to grow and form a self-supporting cellular construct within the mould; removing the self-supporting cellular

construct having a central opening and an outer dimension of the mould from the mould; repeating the steps as described above to obtain a plurality of self-supporting cellular construct having a central opening; placing each of the plurality of self-supporting cellular constructs having a central opening in a series adjacent to one another such that the central opening of each of the self-supporting cellular constructs having a central opening is aligned to one another to thereby form a continuous channel within its central cavity.

[0006] Also described herein is a method of making a self-supporting cellular construct, the method comprises the steps of providing a mould of a desired shape, wherein the mould comprises an area capable of housing a plurality of cells and a removable material within the mould; inserting a plurality of cells in a cell culture medium into the mould; inserting a removable material before or after the previous step; allowing the cells to grow and form a self-supporting cellular structure surrounding the removable material within the mould; and removing the self-supporting cellular structure from the mould to thereby form a self-supporting cellular construct.

[0007] Also described herein is a material-free cellular construct as described herein.

[0008] Also described herein is a material-free tubular cellular construct as described herein.

[0009] In another aspect, the present invention refers to a method of testing chemical agent comprising the steps of providing the cellular construct as described herein; and providing the chemical agent through the channel of the cellular construct and observing changes to the plurality of cells of the cellular constructs.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0011] Fig. 1 shows an assembly of cellular units used to form 3D cellular structures. (a) – (c) are images showing the formation of spheroids consisting of a mixture of hepatocarcinoma cells (hepG2) and endothelial cells (EndoGFP). (d) – (f) are images showing the formation of endothelial tube on a biodegradable suture in a mould, such as a PDMS mould. (g) – (i) are images showing the assembly of the cellular spheroids on an

Claims

1. A method of making a self-supporting cellular construct having a continuous channel within its central cavity, wherein the method comprises the steps of:

a. providing a mould with a central opening, wherein the mould encloses a volume around the central opening (hole) capable of housing a plurality of cells;

b. inserting a plurality of cells in a cell culture medium into the mould;

c. allowing the cells to grow and form a self-supporting cellular construct within the mould;

d. removing the self-supporting cellular construct having a central opening and an outer dimension of the mould from the mould;

e. repeating a to d to obtain a plurality of self-supporting cellular construct having a central opening;

f. placing each of the plurality of self-supporting cellular constructs having a central opening in a series adjacent to one another such that the central opening of each of the self-supporting cellular constructs having a central opening is aligned to one another to thereby form a continuous channel within its central cavity,

wherein the plurality of cells comprises at least two cell types, wherein one cell type is capable of forming cell aggregates and the other cell type is not capable of forming cell aggregates by itself.

2. The method of claim 1, wherein a removable material is threaded through the central opening of the plurality of self-supporting cellular construct having a central opening, thus forming a series of self-supporting cellular construct.

3. The method of claim 2, wherein i) the removable material is removed by physically drawing the removable material away from the plurality of cells formed as a tube; or ii) the removable material is a metal needle or a stick; or iii) the removable material is coated with non-sticking agent.

4. The method of claim 2 or 3, wherein the removable material is removed by biodegradation; optionally the removable material is a biodegradable suture.

5. The method of any one of claims 1 to 4, wherein
the two cell types are provided with a ratio of about 1: about 1, about 2: about 1, about 3: about 1, about 4: about 1, about 5: about 1, about 6: about 1, about 7: about 1, or about 8: about 1 of the cell type that forms self-supporting structure to the other cell type that does not form self-supporting structure.

6. The method of any one of claims 1 to 5, wherein iii) the plurality of cells further comprises parenchymal cells; or iv) wherein the plurality of cell comprises at least one cell type selected from the group consisting of human umbilical vascular endothelial cells (HUVEC), human coronary artery smooth muscle cells (CASMC), human mesenchymal stem cells (hMSC) and hepatocarcinoma cells; optionally
wherein the plurality of cell comprises human umbilical vascular endothelial cells (HUVEC) and any one of human mesenchymal stem cells (hMSC) or human coronary artery smooth muscle cells (CASMC), optionally
wherein the plurality of cell comprising human umbilical vascular endothelial cells (HUVEC) and human mesenchymal stem cells (hMSC) are provided in a ratio that would allow the self-supporting structure to remain stable and do not disintegrate.

7. The method of any one of claims 1 to 6, wherein the cellular construct further comprises at least one capillary that traverses the plurality of cells from the central opening to thereby act as a vascular supply to the plurality of cells in the cellular construct; optionally
wherein the plurality of cells comprises at least one parenchymal cell and the at least one capillary is adjacent to at least one parenchymal cell thereby allowing fluid connection of solution from the central opening to the parenchymal cell.

8. A method of making a self-supporting cellular construct, the method comprises the steps of:
   a. providing a mould of a desired shape, wherein the mould comprises an area capable of housing a plurality of cells and a removable material within the mould;
   b. inserting a plurality of cells in a cell culture medium into the mould;

c. inserting a removable material before or after (b);
d. allowing the cells to grow and form a self-supporting cellular structure surrounding the removable material within the mould; and
e. removing the self-supporting cellular structure from the mould to thereby form a self-supporting cellular construct

wherein the plurality of cells comprises at least two cell types, wherein one cell type is capable of forming cell aggregates and the other cell type is not capable of forming cell aggregates by itself.

9. The method of claim 8, further comprising the step of removing the removable material from the self-supporting cellular structure to thereby form the self-supporting cellular construct with a central channel along the self-supporting cellular construct; optionally
wherein the removable material is removed by physically drawing the removable material away from the plurality of cells formed as a tube; optionally
wherein the removable material is removed by biodegradation; optionally
wherein the removable material is a biodegradable suture.

10. The method of any one of claims 8 to 9, wherein the plurality of cells is selected from the group consisting of endothelial cells, kidney tubule cells, and epithelial cells; optionally
wherein the plurality of cells further comprises parenchymal cells.

11. The method of any one of claims 8 to 10, wherein the self-supporting cellular construct further comprises at least one capillary that connects or traverses the self-supporting cellular construct from central channel to an outer dimension of the self-supporting cellular construct; optionally
wherein the plurality of cells mimics hair growth wherein the removable material is a guide for the growth of the hair shaft.

12. The method of claim 8, wherein the mould is a multi-welled mould to thereby obtain a plurality of self-supporting cellular structures attached to the removable material; optionally

further repeating steps (a) to (e) of claim 8 to thereby obtain a plurality of self-supporting cellular structures on a plurality of removable materials; optionally

wherein the plurality of self-supporting cellular structure on the plurality of removable materials are aligned, or weaved, or braided, or knitted, or knotted.

13. The method of any one of the preceding claims, wherein the mould is made of an elastomeric polymer.

14. The method of claim 13, wherein the elastomeric polymer is selected from the group consisting of agarose, polyethylene glycol gels, poly(2-hydroxyethyl methacrylate) gels (or poly (HEMA) gels), silicon rubbers, polydimethylsiloxane (PDMS), urethane rubbers and ethylene-vinyl acetate (EVA) rubbers.

15. The method of any one of claims 8 to 11, wherein the self-supporting cellular construct further comprises at least one capillary connecting the central channel of the pipe-like cellular construct to the plurality of cells of the cellular construct having a central opening to thereby act as a vascular network connecting the central channel of the pipe-like cellular construct to the plurality of cells in the cellular construct having a central opening.

16. The method of any one of the preceding claims, wherein the cellular construct has substantially circular or elliptical cross section; optionally

wherein the cellular construct forms a cellular O ring with an inner diameter of 5cm, and wall thickness of 500 μm; or wherein the cellular construct is 3 cm in diameter.

17. A method of testing chemical agent comprising the steps of:

a. providing the cellular construct as claimed in any one of claims 1 to 16; and

b. providing the chemical agent through the channel of the cellular construct and

c. observing changes to the plurality of cells of the cellular constructs.

18. The method of claim 17, wherein the changes are differential expression of peptide or gene expression as compared to non-treated control.

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19. The method of any one of claims 17 to 18, wherein the cellular constructs of c are the pipe-like cellular construct and/or cellular construct having a central opening.