ENGINEERED THREE-DIMENSIONAL CONNECTIVE TISSUE CONSTRUCTS AND METHODS OF MAKING THE SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Application Serial No. 61/661,768, filed June 19, 2012, and is a continuation of U.S. Application Serial No. 13/801,780, filed March 13, 2013, each of which is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] A number of pressing problems confront the healthcare industry. As of June 2012 there were 114,636 patients registered by United Network for Organ Sharing (UNOS) as needing an organ transplant. According to UNOS, between January and March 2012 only 6,838 transplants were performed. Each year more patients are added to the UNOS list than transplants are performed, resulting in a net increase in the number of patients waiting for a transplant.

[0003] Additionally, the research and development cost of a new pharmaceutical compound is approximately $1.8 billion. *See* Paul, et al. (2010). How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nature Reviews Drug Discovery* 9(3):203–214. Drug discovery is the process by which drugs are discovered and/or designed. The process of drug discovery generally involves at least the steps of: identification of candidates, synthesis, characterization, screening, and assays for therapeutic efficacy. Despite advances in technology and understanding of biological systems, drug discovery is still a lengthy, expensive, and inefficient process with low rate of new therapeutic discovery.

SUMMARY OF THE INVENTION

[0004] In one aspect, there is provided an engineered, living, three-dimensional connective tissue construct comprising: connective tissue cells cohered to one another to provide a living, three-dimensional connective tissue construct; wherein the construct is bone and is free of pre-formed scaffold.

[0004a] In one aspect, there is provided an array of engineered, living, three-dimensional connective tissue constructs, wherein each construct is an engineered, living, three-dimensional connective tissue construct comprising: connective tissue cells cohered to one another to provide a living, three-dimensional connective tissue construct; wherein the construct is bone and is free of pre-formed scaffold.

[0004b] In one aspect, there is provided a method of fabricating a living, three-dimensional connective tissue construct comprising: incubating a bio-ink, comprising multi-potent cells that have been deposited on a support and exposed to one or more differentiation signals, to allow the bio-ink to cohere and to form a living, three-dimensional connective tissue construct, wherein the construct is bone, wherein said

incubation has a duration of about 1 hour to about 30 days, and wherein the support is not a pre-formed scaffold and the tissue construct is free of pre-formed scaffold

[0004c] In one aspect, disclosed herein are engineered, living, three-dimensional connective tissue constructs comprising: connective tissue cells cohered to one another to provide a living, three-dimensional connective tissue construct; wherein the construct is substantially free of pre-formed scaffold. In some embodiments, the construct is substantially free of any pre-formed scaffold at the time of use. In some embodiments, the construct is non-innervated. In some embodiments, the connective tissue cells comprise connective tissue cells derived *in vitro* from multi-potent cells. In some embodiments, the multi-potent cells comprise one or more of: tissue-specific progenitors, mesenchymal stem/stromal cells, induced pluripotent stem cells, and embryonic stem cells. In some embodiments, the multi-potent cells are derived from mammalian adipose tissue. In other embodiments, the multi-potent cells are derived from mammalian bone marrow. In yet other embodiments, the multi-potent cells are derived from a non-adipose, non-bone marrow tissue

CLAIMS

WHAT IS CLAIMED IS:

1. An engineered, living, three-dimensional connective tissue construct comprising: connective tissue cells cohered to one another to provide a living, three-dimensional connective tissue construct; wherein the construct is substantially free of pre-formed scaffold at the time of use.

2. The construct of claim 1, wherein the construct is non-innervated.

3. The construct of claim 1, wherein the connective tissue cells comprise connective tissue cells derived *in vitro* from multi-potent cells.

4. The construct of claim 3, wherein the multi-potent cells comprise one or more of: tissue-specific progenitors, mesenchymal stem/stromal cells, induced pluripotent stem cells, and embryonic stem cells.

5. The construct of claim 3, wherein the multi-potent cells are derived from mammalian adipose tissue.

6. The construct of claim 3, wherein the multi-potent cells are derived from mammalian bone marrow.

7. The construct of claim 3, wherein the multi-potent cells are derived from a non-adipose, non-bone marrow tissue source.

8. The construct of claim 3, wherein the multi-potent cells were exposed to one or more differentiation signals before fabrication of the construct.

9. The construct of claim 3, wherein the multi-potent cells were exposed to one or more differentiation signals during fabrication of the construct.

10. The construct of claim 3, wherein the multi-potent cells were exposed to one or more differentiation signals after fabrication of the construct.

11. The construct of claim 1, wherein the construct was bioprinted.

12. The construct of claim 11, further comprising an extrusion compound, the extrusion compound improving the suitability of the cells for bioprinting.

13. The construct of claim 1, wherein the connective tissue is selected from the group consisting of: bone, cartilage, tendon, and ligament.

14. The construct of claim 1, further comprising one or more of the following cell types: vascular, endothelial, fibroblasts, pericytes, stem/progenitor cells, immune cells.

15. The construct of claim 1, substantially in the form of a sheet, patch, ring, tube, cube, polyhedron, or sphere.

16. The construct of claim 1, substantially in the form of a shape that mimics the shape or architecture of a native human connective tissue *in vivo*.

17. The construct of claim 1, for implantation in a subject at a site of injury, disease, or degeneration.

18. The construct of claim 1, further comprising one or more of discrete filler bodies, each filler body comprising a biocompatible material, wherein the one or more filler body creates a gap or space in the cohered cells.

19. The construct of claim 18, wherein each filler body substantially resists migration and ingrowth of cells.

20. An array of engineered, living, three-dimensional connective tissue constructs, each construct fabricated by a process comprising: exposing multi-potent cells to one or more differentiation signals to provide a living, three-dimensional connective tissue construct; wherein each connective tissue construct is substantially free of pre-formed scaffold at the time of use; wherein each connective tissue construct is maintained in culture.

21. The array of claim 20, wherein each construct is non-innervated.

22. The array of claim 20, wherein the multi-potent cells comprise one or more of: tissue-specific progenitors, mesenchymal stem/stromal cells, induced pluripotent stem cells, and embryonic stem cells.

23. The array of claim 20, wherein the multi-potent cells are derived from mammalian adipose tissue.

24. The array of claim 20, wherein the multi-potent cells are derived from mammalian bone marrow.

25. The array of claim 20, wherein the multi-potent cells are derived from a non-adipose, non-bone marrow tissue source.

26. The array of claim 20, wherein the multi-potent cells were exposed to the one or more differentiation signals before fabrication of the construct.

27. The array of claim 20, wherein the multi-potent cells were exposed to the one or more differentiation signals during fabrication of the construct.

28. The array of claim 20, wherein the multi-potent cells were exposed to the one or more differentiation signals after fabrication of the construct.

29. The array of claim 20, wherein each construct was bioprinted.

30. The array of claim 20, wherein the connective tissue is selected from the group consisting of: bone, cartilage, tendon, and ligament.

31. The array of claim 20, wherein one or more connective tissue constructs further comprises one or more of the following cell types: endothelial cells, fibroblasts, stem/progenitor cells, pericytes, satellite cells, or vascular cells.

32. The array of claim 20, wherein one or more connective tissue constructs are compound tissue constructs comprising one or more connective tissues.

33. The array of claim 32, wherein one or more connective tissue constructs are compound tissue constructs comprising connective tissue and a non-connective tissue.

34. The array of claim 33, wherein one or more connective tissue constructs are compound tissue constructs comprising bone tissue and a non-connective tissue.

35. The array of claim 20, for use in *in vitro* assays.

36. The array of claim 35, for use in one or more of: drug discovery, drug testing, toxicology testing, disease modeling, three-dimensional biology studies, and cell screening.

37. The array of claim 20, wherein the one or more differentiation signals comprise mechanical, biomechanical, soluble, or physical signals, or combinations thereof.

38. The array of claim 20, wherein one or more constructs further comprises one or more discrete filler bodies, each filler body comprising a biocompatible material, wherein the one or more filler body creates a gap or space in the cohered cells.

39. The array of claim 38, wherein each filler body substantially resists migration and ingrowth of cells.

40. A method of fabricating a living, three-dimensional connective tissue construct comprising: incubating a bio-ink, comprising multi-potent cells that have been deposited on a support and exposed to one or more differentiation signals, to allow the bio-ink to cohere and to

form a living, three-dimensional connective tissue construct, wherein said incubation has a duration of about 1 hour to about 30 days.

41. The method of claim 40, wherein the multi-potent cells comprise one or more of: mesenchymal stem/stromal cells, induced pluripotent stem cells, and embryonic stem cells.

42. The method of claim 40, wherein the multi-potent cells are derived from mammalian adipose tissue.

43. The method of claim 40, wherein the multi-potent cells are derived from mammalian bone marrow.

44. The method of claim 40, wherein the multi-potent cells are derived from a non-adipose, non-bone marrow tissue source.

45. The method of claim 40, wherein the connective tissue cells are exposed to one or more differentiation signals at one or more time intervals between about 1-21 days before depositing the bio-ink onto the support to about 1-21 days after depositing the bio-ink onto the support.

46. The method of claim 40, wherein the bio-ink is deposited by bioprinting.

47. The method of claim 40, wherein the construct is substantially free of any pre-formed scaffold at the time of use.

48. The method of claim 40, wherein the construct is non-innervated.

49. The method of claim 40, wherein the connective tissue is selected from the group consisting of: bone, cartilage, tendon, and ligament.

50. The method of claim 40, wherein the bio-ink further comprises one or more of the following cell types: vascular, endothelial, fibroblasts, pericytes, stem/progenitor cells, immune cells.

51. The method of claim 40, wherein the bio-ink further comprises an extrusion compound.

52. The method of claim 40, wherein the one or more differentiation signals comprise mechanical, biomechanical, soluble, or physical signals, or combinations thereof.

53. The method of claim 40, further comprising depositing one or more discrete filler bodies, each filler body comprising a biocompatible material, wherein the one or more filler body creates a gap or space in the cohered cells.

54. The method of claim 53, wherein each filler body substantially resists migration and ingrowth of cells.

55. The method of claim 40, further comprising assembling a plurality of living, three-dimensional connective tissue constructs into an array by spatially confining the constructs onto or within a biocompatible surface.

56. The method of claim 40, wherein the construct is suitable for implantation in a subject at a site of injury, disease, or degeneration.

CLAIMS

WHAT IS CLAIMED IS:

1. An engineered, living, three-dimensional connective tissue construct comprising: connective tissue cells cohered to one another to provide a living, three-dimensional connective tissue construct; wherein the construct is bone and is free of pre-formed scaffold.

2. The construct of claim 1, wherein the construct is non-innervated.

3. The construct of claim 1, wherein the connective tissue cells comprise connective tissue cells derived *in vitro* from multi-potent cells.

4. The construct of claim 3, wherein the multi-potent cells comprise one or more of: tissue-specific progenitors, mesenchymal stem/stromal cells, induced pluripotent stem cells, and embryonic stem cells.

5. The construct of claim 3, wherein the multi-potent cells are derived from mammalian adipose tissue.

6. The construct of claim 3, wherein the multi-potent cells are derived from mammalian bone marrow.

7. The construct of claim 3, wherein the multi-potent cells are derived from a non-adipose, non-bone marrow tissue source.

8. The construct of claim 3, wherein the multi-potent cells were exposed to one or more differentiation signals before fabrication of the construct.

9. The construct of claim 3, wherein the multi-potent cells were exposed to one or more differentiation signals during fabrication of the construct.

10. The construct of claim 3, wherein the multi-potent cells were exposed to one or more differentiation signals after fabrication of the construct.

11. The construct of claim 1, wherein the construct was bioprinted.

12. The construct of claim 11, further comprising an extrusion compound, the extrusion compound improving the suitability of the cells for bioprinting.

13. The construct of claim 1, substantially in the form of a sheet, patch, ring, tube, cube, polyhedron, or sphere.

14. The construct of claim 1, substantially in the form of a shape that mimics the shape or architecture of a native human connective tissue *in vivo*.

15. The construct of claim 1, for implantation in a subject at a site of injury, disease, or degeneration.

16. The construct of claim 1, further comprising one or more of discrete filler bodies, each filler body comprising a biocompatible material.

17. An array of engineered, living, three-dimensional connective tissue constructs, wherein each construct is an engineered, living, three-dimensional connective tissue construct comprising: connective tissue cells cohered to one another to provide a living, three-dimensional connective tissue construct; wherein the construct is bone and is free of pre-formed scaffold.

18. The array of claim 17, wherein each construct is non-innervated.

19. The array of claim 17, wherein the connective tissue cells in each construct comprise connective tissue cells derived in vitro from multi-potent cells.

20. The array of claim 19, wherein the multi-potent cells comprise one or more of: tissue-specific progenitors, mesenchymal stem/stromal cells, induced pluripotent stem cells, and embryonic stem cells.

21. The array of claim 19, wherein the multi-potent cells are derived from mammalian adipose tissue.

22. The array of claim 19, wherein the multi-potent cells are derived from mammalian bone marrow.

23. The array of claim 19, wherein the multi-potent cells are derived from a non-adipose, non-bone marrow tissue source.

24. The array of claim 19, wherein the multi-potent cells were exposed to one or more differentiation signals before fabrication of the construct.

25. The array of claim 19, wherein the multi-potent cells were exposed to one or more differentiation signals during fabrication of the construct.

26. The array of claim 19, wherein the multi-potent cells were exposed to one or more differentiation signals after fabrication of the construct.

27. The array of claim 17, wherein each construct was bioprinted.

28. The array of claim 17, for use in *in vitro* assays.

29. The array of claim 28, for use in one or more of: drug discovery, drug testing, toxicology testing, disease modeling, three-dimensional biology studies, and cell screening.

30. The array of any one of claims 24-26, wherein the one or more differentiation signals comprise mechanical, biomechanical, soluble, or physical signals, or combinations thereof.

31. The array of claim 17, wherein one or more constructs further comprises one or more discrete filler bodies, each filler body comprising a biocompatible material.

32. A method of fabricating a living, three-dimensional connective tissue construct comprising: incubating a bio-ink, comprising multi-potent cells that have been deposited on a support and exposed to one or more differentiation signals, to allow the bio-ink to cohere and to form a living, three-dimensional connective tissue construct, wherein the construct is bone, wherein said incubation has a duration of about 1 hour to about 30 days, and wherein the support is not a pre-formed scaffold and the tissue construct is free of pre-formed scaffold.

33. The method of claim 32, wherein the multi-potent cells comprise one or more of: mesenchymal stem/stromal cells, induced pluripotent stem cells, and embryonic stem cells.

34. The method of claim 32, wherein the multi-potent cells are derived from mammalian adipose tissue.

35. The method of claim 32, wherein the multi-potent cells are derived from mammalian bone marrow.

36. The method of claim 32, wherein the multi-potent cells are derived from a non-adipose, non-bone marrow tissue source.

37. The method of claim 32, wherein the connective tissue cells are exposed to one or more differentiation signals at one or more time intervals between about 1-21 days before depositing the bio-ink onto the support to about 1-21 days after depositing the bio-ink onto the support.

38. The method of claim 32, wherein the bio-ink is deposited by bioprinting.

39. The method of claim 32, wherein the construct is non-innervated.

40. The method of claim 32, wherein the bio-ink further comprises an extrusion compound.

41. The method of claim 32, wherein the one or more differentiation signals comprise mechanical, biomechanical, soluble, or physical signals, or combinations thereof.

42. The method of claim 32, further comprising depositing one or more discrete filler bodies, each filler body comprising a biocompatible material.

43. The method of claim 32, further comprising assembling a plurality of living, three-dimensional connective tissue constructs into an array by spatially confining the constructs onto or within a biocompatible surface.

44. The method of claim 32, wherein the construct is suitable for implantation in a subject at a site of injury, disease, or degeneration.