

WHAT IS CLAIMED IS:

1. A container for the generation of bioengineered tissue, where the generation comprises introducing epithelial and mesenchymal cells into or onto a biomatrix scaffold, wherein the biomatrix scaffold comprises collagens.
2. The container of claim 1, in which epithelial and mesenchymal cells are maturational lineage partners.
3. The container of claim 1, in which epithelial and mesenchymal cells are in a seeding medium, and the seeding medium is replaced with a differentiation medium after an initial incubation period.
4. The container of claim 3, where in the differentiation medium comprises:
 - a. A basal medium,
 - b. Lipids, insulin, transferrin, antioxidants,
 - c. Copper,
 - d. Calcium,
 - e. One or more signals for the propagation or maintenance of epithelial cells, and/or
 - f. One or more signals for the propagation or maintenance of mesenchymal cells.
5. The container of claim 3 in which the seeding medium is serum-free or is supplemented with between about 2% to 10% fetal serum, optionally over the duration of a few hours.
6. The container of claim 3, where in the seeding medium comprises:
 - a. A basal medium
 - b. Lipids

- c. Insulin
 - d. Transferrin
 - e. Antioxidants.
7. The container of claim 3 in which the epithelial and mesenchymal cells in the seeding medium is incubated at 4°C in the seeding medium for 4 to 6 hours prior to introduction into the biomatrix scaffolds
8. The container of claim 1, in which the biomatrix scaffold is three-dimensional
9. The container of claim 1, in which the collagens in the biomatrix scaffold comprise (i) nascent collagens, (ii) aggregated but not cross-linked collagen molecules, (iii) cross-linked collagens.
10. The container of claim 10 in which the epithelial and mesenchymal cells in the seeding medium are introduced in multiple intervals, each interval followed by a period of rest.
11. The container of claim 10 in which the interval is about 10 minutes and the period of rest is about 10 minutes.
12. The container of claim 10 in which the seeding density is up to about 12 million cells per gram of wet weight of the biomatrix scaffolds and introduced during one or more intervals.
13. The container of claim 10 in which the epithelial and mesenchymal or non-parenchymal cells in the seeding medium are introduced at a rate of ~15 ml/min for one or more intervals.
14. The container of claim 10, in which the epithelial and mesenchymal cells in the seeding medium are introduced in 10 minute intervals, each followed by a 10 minute period of rest.
15. The container of claim 10 in which the epithelial and mesenchymal cells in the seeding medium are is introduced at a rate of 1.3 ml/min after three intervals.

16. The container of claim 1 in which the epithelial and mesenchymal cells comprise cells isolated from a fetal or neonatal organ.
17. The container claim 1 in which the epithelial and mesenchymal cells comprise cells isolated from an adult or child donor
18. The container of claim 1 in which the epithelial and mesenchymal cells comprise:
- a. epithelial cells comprising one or more of stem cells, committed progenitors, diploid adult cells, polyploid adult cells, and/or terminally differentiated cells
 - and/or
 - b. mesenchymal cells comprising one or more of angioblasts, precursors to endothelia, mature endothelia, precursors to stellate cells, mature stellate cells, precursors to stroma, mature stroma, smooth muscle cells, precursors to hematopoietic cells, and/or mature hematopoietic cells.
19. The container of claim 1 in which the epithelial and mesenchymal cells comprise:
- a. epithelial cells comprising one or more of biliary tree stem cells, gall bladder- derived stem cells, hepatic stem cells, hepatoblasts, committed hepatocytic and biliary progenitors, axin2+ progenitors (such as axin2+ hepatic progenitors), mature parenchymal cells (such as hepatocytes, cholangiocytes) , pancreatic stem cells, and pancreatic committed progenitors, islet cells, and/or acinar cells, and/or
 - b. mesenchymal cells comprising one or more of angioblasts, stellate cell precursors, stellate cells, mesenchymal stem cells, pericytes, smooth muscle cells, stromal cells, endothelial cell precursors, endothelial cells, hematopoietic cell precursors, and/or hematopoietic cells.
20. The container of claim 1 in which the epithelial cells comprises one or more of stem cells and their descendants from the biliary tree, liver, pancreas, hepato-pancreatic common duct, and/or gall bladder and/or mesenchymal cells comprising one or more of angioblasts, precursors to endothelia and stellate cells, mesenchymal stem cells, stellate cells, stroma,

smooth muscle cells, endothelia, bone marrow-derived stem cells, hematopoietic cell precursors, and/or hematopoietic cells.

21. The container claim 1 in which the epithelial and mesenchymal cells consists of about 80% epithelial and 20% mesenchymal respectively

22. The container of claim 1 in which the epithelial and mesenchymal cells comprise at least 50% stem cells and/or precursor cells.

23. The container of claim 1, wherein the epithelial and mesenchymal cells do not comprise any terminally differentiated hepatocytes and/or pancreatic cells.

24. The container of claim 1 in which the biomatrix scaffold comprises one or more collagen associated matrix components comprising one or more of laminins, nidogen, elastins, proteoglycans, hyaluronans, non-sulfated glycosaminoglycans, sulfated glycosaminoglycans, growth factors and/or cytokines associated with the matrix components.

25. The container of claim 1 in which the biomatrix scaffold comprises greater than 20-50% of matrix-bound signaling molecules found in vivo.

26. The container of claim 1 in which the biomatrix scaffold comprises a matrix remnant of the vascular tree of the tissue and/or wherein the matrix remnant provides vascular support of the cells in the bioengineered tissue

27. A three-dimensional scaffold comprising extracellular matrix, which in turn comprises (i) native collagens found in an organ and/or (ii) matrix remnants of a vascular tree found in an organ

28. A three-dimensional micro-organ generated in the container of claim 1.

29. A bioengineered tissue comprising zonation-dependent phenotypic traits characteristic of native liver, said phenotypic traits including (a) periportal region having traits of stem/progenitors, diploid adult cells and/or associated mesenchymal precursor cells, (b) a mid-acinar region having cells with traits of sinusoidal plates of mature parenchymal cells and mesenchymal cells, and/or (c) a pericentral region having traits of

terminally differentiated epithelial and, apoptotic cells associated with fenestrated endothelia and/or axin2⁺ hepatic progenitors that are connected to endothelia of the central vein.

30. The bioengineered tissue of claim 29 in which the phenotypic traits further include traits associated with diploid epithelial cells and/or mesenchymal cells of the periportal zone

31. The bioengineered tissue of claim 29 in which the phenotypic traits further include traits of mature epithelial cells and/or mesenchymal cells found in the mid-acinar region of native liver.

32. The bioengineered tissue of claim 29 in which the phenotypic traits further include traits of epithelial or parenchymal and/or mesenchymal cells of the pericentral zone.

33. The bioengineered tissue of claim 29 further comprising: (i) polyploid hepatocytes associated with fenestrated endothelial cells, and/or (ii) diploid hepatic progenitors (such as axin2⁺ cells) connected to endothelia of a central vein

34. The bioengineered tissue of claim 29 in which the periportal region is enriched in traits of the stem/progenitor cell niches that comprise hepatic stem cells, hepatoblasts, committed progenitors, and/or diploid adult hepatocytes.

35. The bioengineered tissue of claim 29 in which the epithelial and mesenchymal cells further comprise epithelial cells comprising precursors and/or mature forms of hepatocytes and/or cholangiocytes.

36. The bioengineered tissue of claim 29 in which the epithelial and mesenchymal cells further comprise mesenchymal cells comprising precursors and/or mature forms of stellate cells, pericytes, smooth muscle cells, stroma, endothelia and/or hematopoietic cells

37. A three-dimensional micro-organ comprised of the bioengineered tissue of claim 29.

38. The three-dimensional micro-organ of claim 37 generated in the container of claim 1.

39. A kit for culturing the micro-organ in the container of claim 1 with accompanying instructions.
40. A method of evaluating a treatment for an organ comprising administering the treatment to a bioengineered tissue or three-dimensional micro-organ of claim 29.
41. A differentiation medium for both epithelial and mesenchymal cells comprising
- a. A basal medium containing lipids, insulin, transferrin, antioxidants,
 - b. Copper,
 - c. Calcium,
 - d. One or more signals for the propagation and/or maintenance of epithelial cells, and/or
 - e. One or more signals for the propagation and/or maintenance of mesenchymal cells.
42. The differentiation medium of claim 41 in which the basal medium is Kubota's Medium.
43. The differentiation medium of claim 41 further comprising one or more lipid binding proteins.
44. The differentiation medium of claim 43 in which the one or more lipid binding proteins is high-density lipoprotein (HDL).
45. The differentiation medium of claim 41 further comprising one or more purified fatty acids.
46. The differentiation medium of claim 45 in which the one or more purified fatty acids comprises palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, and/or linolenic acid.
47. The differentiation medium of claim 41 further comprising one or more sugars.

48. The differentiation medium of any one of claim 47 in which the one or more sugars comprises galactose, glucose, and/or fructose.
49. The differentiation medium of claim 41 further comprising one or more glucocorticoids.
50. The differentiation medium of claim 49 in which the one or more glucocorticoids comprises dexamethasone and/or hydrocortisone
51. A bioengineered tissue comprising zonation-dependent phenotypic traits characteristic of native pancreas and/or that includes zonation associated with pancreatic cells in the head of the pancreas and/or those associated with pancreatic cells in the tail of the pancreas.