Claims

1. A method for the generation of a bioengineered ~~tissue~~ liver in a container, where the generation comprises introducing epithelial and mesenchymal cells, wherein the epithelial and mesenchymal cells are maturational lineage partners, into or onto a biomatrix scaffold, wherein the biomatrix scaffold comprises collagens, wherein the biomatrix scaffold is preferably three-dimensional, wherein the epithelial and mesenchymal cells are in a seeding medium, and the seeding medium is replaced with a differentiation medium after an initial incubation period, wherein the differentiation medium comprises a basal medium, and wherein the differentiation medium further comprises human growth hormone, vascular endothelial growth factor, insulin-like growth factor, and angiopoietin, wherein the bioengineered liver comprises zonation-dependent phenotypic traits characteristic of native liver.

2. The method 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 initial incubation period, wherein the differentiation medium preferably comprises:
a) a basal medium;
b) lipids, insulin, transferrin, antioxidants;
c) copper;
d) calcium;
e) a signaling molecule for the propagation or maintenance of epithelial cells; and
f) a signaling molecule for the propagation or maintenance of mesenchymal cells;
preferably in which the seeding medium is serum-free, ~~optionally over the duration of few hours,~~ wherein the seeding medium preferably comprises:
a) a basal medium;
b) lipids;
c) insulin;
d) transferrin; and/or
e) antioxidants.

3. The method of claim 2, 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.

4. The method of any one of claims 1-3, in which the collagens in the biomatrix scaffold comprise
(i) nascent collagens,
(ii) aggregated but not cross-linked collagen molecules, and/or
(iii) cross- linked collagens.

5. The method of claim 2 in which the epithelial and mesenchymal cells in the seeding medium are introduced in one or more intervals, each interval followed by a period of rest, preferably in which the one or more intervals are about 10 minutes and the period of rest is about 10 minutes, preferably in which the epithelial and mesenchymal cells are introduced at a seeding density of up to about 12 million cells per gram of wet weight of the biomatrix scaffold, preferably in which the epithelial and mesenchymal cells in the seeding medium are introduced at a rate of ~15 ml/min for one or more intervals, more preferably 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, more preferably in which the epithelial and mesenchymal cells in the seeding medium are introduced at a rate of 1.3 ml/min after three intervals.

6. The method of any one of claims 1-5, in which the epithelial and mesenchymal cells comprise cells isolated from a fetal or neonatal organ, or in which the epithelial and mesenchymal cells comprise cells isolated from an adult or child donor.

7. The method of any one of claims 1-6, 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; preferably 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, hematopoetic cell precursors, and/or hematopoetic cells, more preferably 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 the mesenchymal cells comprise 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, hematopoetic cell precursors, and/or hematopoetic cells.

8. The method of any one of claims 1-7, in which the epithelial and mesenchymal cells consists of about 80% epithelial cells and 20% mesenchymal cells respectively, or in which the epithelial and mesenchymal cells comprise at least 50% stem cells and/or precursor cells, preferably wherein the epithelial and mesenchymal cells do not comprise any terminally differentiated hepatocytes and/or pancreatic cells.

9. The ~~container~~ method of any one of claims 1-8, 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, or in which the biomatrix scaffold comprises 20-50% of matrix-bound signaling molecules found in vivo, or in which the biomatrix scaffold comprises a matrix remnant of a vascular tree of a tissue that the biomatrix scaffold was derived from, and/or wherein the matrix remnant provides vascular support for the bioengineered tissue.

10. 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, preferably 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,
    (b) a mid-acinar region having cells with traits of sinusoidal plates of mature parenchymal cells and mesenchymal cells, preferably in which the epithelial and mesenchymal cells further comprise epithelial cells comprising precursors and/or mature forms of hepatocytes and/or

cholangiocytes, more preferably 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, and

(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; in which the phenotypic traits optionally further include traits associated with diploid epithelial cells and/or mesenchymal cells of the periportal zone,in which the phenotypic traits optionally further include traits of mature epithelial cells and/or mesenchymal cells found in the mid-acinar region of native liver, in which the phenotypic traits optionally further include traits of epithelial or parenchymal and/or mesenchymal cells of the pericentral zone, preferably 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.

11. A method of evaluating a treatment for an organ comprising administering in vitro the treatment to a bioengineered tissue of claim 10, or to a three-dimensional micro-organ comprising the bioengineered tissue of claim 10.

12. The method of any one of claims 1-9, wherein the differentiation medium further comprises one or more lipid binding proteins, one or more purified fatty acids, one or more sugars, one or more glucocorticoids, nicotinomide, Tri- iodothyronine, epidermal growth factor and/or hepatocyte growth factor.

13. The method of any one of claims 1-9, wherein the basal medium is Kubota's medium.

14. The method of claim 12, wherein the one or more lipid binding proteins is high-density lipoprotein (HDL).

15. The method of claim 12, wherein the one or more purified fatty acids comprises palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, and/or linolenic acid.

16. The method of claim 12, wherein the one or more sugars comprises galactose, glucose, and/or fructose.

17. The method of claim 12, wherein the one or more glucocorticoids comprises dexamethasone and/or hydrocortisone.

18. The method of any one of claims 1-9, wherein the seeding medium is serum-free or is supplemented with between about 2% to 10% fetal serum.