

CLAIMS

1. Bio-printed kidney tissue, wherein the bio-printed kidney tissue is enriched with nephrons which are distributed throughout the tissue.
2. The bio-printed kidney tissue of claim 1, wherein the bio-printed kidney tissue is a layer of bio-printed tissue comprising a surface area of nephron tissue of greater than 0.2 mm^2 per 10,000 cells printed.
3. The bio-printed kidney tissue of claim 1 or 2, wherein the bio-printed kidney tissue is a layer of bio-printed kidney tissue comprising about 30,000 cells per mm^2 or less when printed.
4. The bio-printed kidney tissue of any one of the preceding claims, wherein the bio-printed kidney tissue expresses high levels of any one or more of SULT1E1, SLC30A1, SLC51B, FABP3, HNF4A, CUBN, LRP2, EPCAM and MAFB.
5. The bio-printed kidney tissue of claim 4, wherein the bio-printed kidney tissue comprises nephrons in which the proximal tubule and distal tubule segments express markers of maturation, including HNF4A and SLC12A1.
6. The bio-printed kidney tissue of claim 4 or 5, wherein the bio-printed kidney tissue expresses each of the markers HNF4A, CUBN, LRP2, EPCAM and MAFB.
7. The bio-printed kidney tissue of any one of the preceding claims, wherein the height of the bio-printed kidney tissue is about $50 \text{ }\mu\text{m}$ or less when printed.
8. The bio-printed kidney tissue of any one of the preceding claims, wherein the bio-printed kidney tissue has a length of from about 1 mm to about 30 mm and a width of from about 0.5 mm to about 20 mm.
9. The bio-printed kidney tissue of claim 7 or 8, wherein the bio-printed kidney tissue comprises from about 5 to about 100 nephrons / mm^2 of bio-printed kidney tissue.
10. Bio-printed kidney tissue comprising a predetermined amount of a bio-ink, wherein the bio-ink comprises a plurality of cells, wherein the bio-ink is bio-printed in a layer that is about $50 \text{ }\mu\text{m}$ high or less and wherein the bio-printed bio-ink is induced to form kidney tissue.

11. The bio-printed kidney tissue of claim 10, wherein the bio-ink is bio-printed in a layer selected from about 20 μm high to about 40 μm high.
12. The bio-printed kidney tissue of claim 10, wherein the bio-ink is bio-printed in a layer about 30 μm high.
13. The bio-printed kidney tissue of claim 10, wherein the bio-ink is bio-printed in a layer about 25 μm high.
14. The bio-printed kidney tissue of any one of claims 10 - 14, wherein the predetermined amount of bio-ink comprises between approximately 10,000 cells/ μl and approximately 400,000 cells/ μl .
15. The bio-printed kidney tissue of any one of claims 10 - 14, wherein said plurality of cells comprises partly differentiated cells.
16. The bio-printed kidney tissue of any one of claims 10 - 15, wherein said plurality of cells comprises renal progenitor cells.
17. The bio-printed kidney tissue of claim 16, wherein the renal progenitor cells comprise nephron progenitor cells.
18. The bio-printed kidney tissue of claim 16 or 17, wherein the renal progenitor cells comprise ureteric epithelial progenitor cells.
19. The bio-printed kidney tissue of any one of claims 10 - 15, wherein said plurality of cells comprises intermediate mesoderm cells.
20. The bio-printed kidney tissue of any one of claims 10 - 15, wherein said plurality of cells comprises metanephric mesenchyme cells.
21. The bio-printed kidney tissue of any one of claims 10 - 15, wherein said plurality of cells comprises nephric duct cells.

22. The bio-printed kidney tissue of any one of claims 10 - 15, wherein said plurality of cells comprises fully differentiated cells.
23. The bio-printed kidney tissue of any one of claims 10 - 22, wherein said plurality of cells comprises patient-derived cells.
24. The bio-printed kidney tissue of any one of claims 10 - 23, wherein said plurality of cells comprises cells from a reporter cell line.
25. The bio-printed kidney tissue of any one of claims 10 - 24, wherein said plurality of cells comprises gene-edited cells.
26. The bio-printed kidney tissue of any one of claims 10 - 25, wherein said plurality of cells comprises diseased cells, healthy cells, or a combination of diseased and healthy cells.
27. The bio-printed kidney tissue of any one of claims 10 - 26, wherein the bio-printed kidney tissue comprises a surface area of nephron tissue of greater than 0.2 mm² per 10,000 cells printed.
28. The bio-printed kidney tissue of any one of claims 10 - 27, wherein the bio-printed kidney tissue comprises about 30,000 cells per mm² or less when printed.
29. The bio-printed kidney tissue of any one of claims 10 - 28, wherein the bio-printed kidney tissue expresses high levels of any one or more of HNF4A, CUBN, LRP2, EPCAM and MAFB.
30. The bio-printed kidney tissue of claim 29, wherein the bio-printed kidney tissue comprises nephrons in which the proximal tubule and distal tubule segments express markers of maturation, including HNF4A.
31. The bio-printed kidney tissue of claim 29 or 30, wherein the bio-printed kidney tissue expresses each of the markers HNF4A, CUBN, LRP2, EPCAM and MAFB.
32. The bio-printed kidney tissue of any one of claims 1 - 31, wherein the tissue comprises from about 5 to about 100 nephrons / 10,000 cells printed.

33. The bio-printed kidney tissue of any one of claims 10 - 32, wherein the tissue has an even distribution of nephrons throughout the bio-printed layer.
34. The bio-printed kidney tissue of any one of claims 10 - 33, wherein the tissue has an even distribution of glomerular structures expressing MAFB throughout the bio-printed layer.
35. The bio-printed kidney tissue of any one of claims 1 - 34, further comprising a bio-compatible scaffold.
36. The bio-printed kidney tissue of claim 35, wherein bio-ink is bio-printed onto a bio-compatible scaffold.
37. The bio-printed kidney tissue of any one of claims 35 or 36, wherein the biocompatible scaffold is a hydrogel.
38. The bio-printed kidney tissue of any one of claims 35 - 37, wherein the biocompatible scaffold is biodegradable or bio-absorbable.
39. The bio-printed kidney tissue of any one of claims 10 - 38, wherein the bio-ink further comprises one or more bioactive agents.
40. The bio-printed kidney tissue of claim 39, wherein said one or more bioactive agents promotes induction of kidney tissue from said plurality of cells.
41. A method for producing bio-printed kidney tissue comprising the steps of: bio-printing a pre-determined amount of a bio-ink onto a surface, wherein the bio-ink comprises a plurality of cells, and wherein the bio-ink is bio-printed in a layer that is about 50 μm high or less; and inducing the bio-printed, pre-determined amount of the bio-ink to form bio-printed kidney tissue.
42. The method of claim 41, wherein at the step of bio-printing the bio-ink is bio-printed in a layer selected from about 20 μm high to about 40 μm high.
43. The method of claim 41, wherein at the step of bio-printing, wherein the bio-ink is bio-printed in a layer about 30 μm high.

44. The method of claim 41, wherein at the step of bio-printing the bio-ink is bio-printed in a layer about 25 μm high.
45. The method of any one of claims 41 - 44, wherein the predetermined amount of bio-ink comprises between approximately 10,000 cells/ μl and approximately 400,000 cells/ μl .
46. The method according to claim 45, wherein the bio-ink comprises about 200,000 cells/ μl .
47. The method of any one of claims 41 - 46, wherein said plurality of cells comprises partly differentiated cells.
48. The method of any one of claims 41 - 46, wherein said plurality of cells comprises renal progenitor cells.
49. The bio-printed kidney tissue of claim 48, wherein the renal progenitor cells comprise nephron progenitor cells.
50. The method of claim 48 or 49, wherein the renal progenitor cells comprise ureteric epithelial progenitor cells.
51. The method of any one of claims 41 - 47, wherein said plurality of cells comprises intermediate mesoderm cells, preferably a culture expanded population of stem cell-derived intermediate mesoderm cells.
52. The method of any one of claims 41 - 47, wherein said plurality of cells comprises metanephric mesenchyme cells.
53. The method of any one of claims 41 - 47, wherein said plurality of cells comprises nephric duct cells.
54. The method of any one of claims 41 - 47, wherein said plurality of cells comprises fully differentiated cells.
55. The method of any one of claims 41 - 54, wherein said plurality of cells comprises patient-derived cells.

56. The method of any one of claims 41 - 55, wherein said plurality of cells comprises cells from a reporter cell line.
57. The method of any one of claims 41 - 56, wherein said plurality of cells comprises gene-edited cells.
58. The method of any one of claims 41 - 57, wherein said plurality of cells comprises diseased cells, healthy cells, or a combination of diseased and healthy cells.
59. The method of any one of claims 41 - 58, wherein the bio-printed kidney tissue comprises from about 5 to about 100 nephrons / 10,000 cells printed.
60. The method of any one of claims 41 - 59, wherein the bio-printed kidney tissue has an even distribution of nephrons throughout the bio-printed layer.
61. The method of any one of claims 41 - 60, wherein the bio-printed kidney tissue has an even distribution of glomerular structures expressing MAFB throughout the bio-printed layer.
62. The method of any one of claims 41 - 61, wherein at the step of bio-printing the bio-ink is bio-printed onto a bio-compatible scaffold.
63. The method of claim 62, wherein the biocompatible scaffold is a hydrogel.
64. The method of any one of claims 62 or 63, wherein the biocompatible scaffold is biodegradable or bio-absorbable.
65. The method of any one of claims 41 - 64, wherein the bio-ink further comprises one or more bioactive agents.
66. The method of claim 65, wherein said one or more bioactive agents promotes induction of kidney tissue from said plurality of cells.
67. The method of any one of claims 41 - 66, wherein the step of inducing comprises contacting the bio-printed, predetermined amount of bio-ink with FGF-9.

68. The method of claim 67, wherein the step of inducing comprises contacting the bio-printed, predetermined amount of bio-ink with FGF-9 for a period of 5 days.
69. The method of any one of claims 41 – 68, wherein the plurality of cells is contacted with a cell culture medium comprising CHIR before being bio-printed.
70. The method of any one of claims 41 – 69, wherein the bio-printing step uses an extrusion-based bio-printer.
71. The method of any one of claims 41 – 70, wherein at the step of bio-printing, a dispensing apparatus of a bio-printer is configured to dispense said layer in one or more lines.
72. The method of any one of claims 41 – 71, wherein at the step of bio-printing, a dispensing apparatus of a bio-printer is configured to dispense said layer in one or more lines so as to form a continuous sheet or patch.
73. Bio-printed kidney tissue produced according to any one of claims 41 – 72.
74. Bio-printed kidney tissue of any one of claims 1 – 40, or 73, for use in the treatment of kidney disease or renal failure in a subject in need thereof.
75. Use of bio-printed kidney tissue of any one of claims 1 – 40, or 73, in the manufacture of a medicament for the treatment of kidney disease in a subject in need thereof.
76. A method of treating kidney disease or renal failure in a subject in thereof, comprising administering to the subject bio-printed kidney tissue of any one of claims 1 – 40, or 73.
77. The bio-printed kidney tissue of any one of claims 1 – 40, or 73, for use according to claim 74, the use of claim 75, or the method of claim 76, wherein in said treatment the bio-printed kidney tissue is transplanted under the renal capsule of said subject.