multicellular structures contain fully segmented nephrons associated with a collecting duct network surrounded by renal interstitium and endothelial cells. They show gene expression equivalent to the human fetal kidney in Trimester 1 of development.

[0005] Despite this important outcome, kidney organoids produced according to previously described methods are self-limiting due to the lack of a sustained nephron progenitor population. During normal human kidney development, ongoing nephron formation occurs from a persisting nephron progenitor population. While it has been shown that this population is present in a kidney organoid, it has also been shown that this population of progenitors generates nephrons and is then lost, limiting the maximal nephron number that can be generated using this approach (Howden et al, EMBO Reports, 2019). A key factor for the generation of maximal functional kidney tissue from stem cells is the relative proportion of the tissue comprised of nephrons. A second key factor is the reduction of unwanted non-renal populations. Accordingly, engineered kidney tissue with a greater number of nephrons per number of cells used to generate such tissue and with more uniform distribution of nephrons is required. A third key factor is kidney tissue in which the component nephrons showed improved patterning and evidence of nephron segment maturation. A fourth key factor to the generation of transplantable renal replacement tissue is a capacity to manufacture such tissue in a reliable and reproducible fashion amenable to automation.

Summary

[0006] The inventors have surprisingly found that an in-vitro engineered, or bio-printed, kidney tissue, derived from a composition comprising stem cell-derived renal progenitor cells, can be generated with an increased or decreased number of nephrons arising per number of cells used to generate the tissue, depending on the spatial parameters applied to the bio-printing of the tissue. It has also been surprisingly found that it is possible to generate bio-printed tissue with more uniform distribution of nephrons. The inventors have surprisingly discovered that by modifying the spatial parameters of bio-printed tissue, more nephrons can be generated from the same amount of starting material (cells), and that the resulting tissue has improved characteristics. Accordingly, described herein are bio-printed kidney tissues enriched for maturing nephrons and methods for producing the same.

[0007] According to a first aspect, the present disclosure provides bio-printed kidney tissue, wherein the bio-printed kidney tissue is enriched with nephrons which are distributed

throughout the tissue. In a preferred example, the nephrons are evenly or uniformly distributed through the printed tissue.

[0008] According to a second example, the present disclosure provides 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 less than about 50 μm high and wherein the bio-printed bio-ink is induced to form kidney tissue. In another example, the height of the bio-printed kidney tissue after the bio-printed bio-ink is induced to form kidney tissue is about 150 μm or less. In other words, the height of the final bio-printed kidney tissue is about 150 μm or less.

[0009] According to a third example, the present disclosure provides bio-printed kidney tissue comprising a predetermined amount of a bio-ink, wherein the bio-ink comprises a plurality of cells and the bio-ink is bio-printed in a layer that comprises about 30,000 cells per mm^2 or less.

[00010] According to another aspect, the present disclosure provides 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 less than about 50 μm high; and inducing the bio-printed, pre-determined amount of the bio-ink to form bio-printed kidney tissue.

[00011] According to a fifth example, the present disclosure provides 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 that are bio-printed in a layer that comprises about 30,000 cells per mm^2 or less; and inducing the bio-printed, pre-determined amount of the bio-ink to form bio-printed kidney tissue.

[00012] In another aspect, the present disclosure provides bio-printed kidney tissue produced according to the method as disclosed herein. According to a sixth example, the present disclosure provides bio-printed kidney tissue produced according to the method of the fourth or fifth examples.

[00013] In another aspect, the present disclosure provides bio-printed kidney tissue as disclosed herein for use in the treatment of kidney disease or renal failure in a subject in need thereof. According to a seventh example, the present disclosure provides bio-printed kidney tissue of any one of the first, second, third or sixth examples, for use in the treatment of kidney disease or renal failure in a subject in need thereof.

[00014] In another aspect, the present disclosure provides use of bio-printed kidney tissue as disclosed herein in the manufacture of a medicament for the treatment of kidney disease in a subject in need thereof. According to an eighth example, the present disclosure provides use of bio-printed kidney tissue of any one of the first, second, third or sixth examples, in the manufacture of a medicament for the treatment of kidney disease in a subject in need thereof.

[00015] According to a ninth example, the present disclosure provides 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 the first, second, third or sixth examples.

[00016] Numbered statements of the disclosure are as follows:

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 statement 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 statement 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 statements, 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 statement 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 statement 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 statements, wherein the height of the bio-printed kidney tissue is about 50 µm or less when printed.

8. The bio-printed kidney tissue of any one of the preceding statements, 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 statement 7 or 8, wherein the bio-printed kidney tissue comprises from about 5 to about 100 nephrons / mm^2 of bio-printed kidney tissue.

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 µm 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 1 mm to 30 mm and a width of from 0.5 mm to 20 mm.

9. The bio-printed kidney tissue of claim 7 or 8, wherein the bio-printed kidney tissue comprises from 5 to 100 nephrons / mm^2 of bio-printed kidney tissue.

10. The bio-printed kidney tissue of any one of claims 1 - 9, wherein the bioprinted kidney tissue comprises a plurality of cells, and the plurality of cells comprises partly differentiated cells, renal progenitor cells, renal progenitor cells further comprising nephron progenitor cells, renal progenitor cells further comprising ureteric epithelial progenitor cells, intermediate

mesoderm cells, metanephric mesenchyme cells, nephric duct cells, fully differentiated cells, patient-derived cells, cells from a reporter cell line, gene-edited cells, and/or diseased cells, healthy cells, or a combination of diseased and healthy cells.

11. The bio-printed kidney tissue of any one of claims 1 - 10, wherein the bio-printed kidney tissue comprises a surface area of nephron tissue of greater than 0.2 mm^2 per 10,000 cells printed.

12. The bio-printed kidney tissue of any one of claims 1 - 11, wherein the bio-printed kidney tissue expresses high levels of any one or more of HNF4A, CUBN, LRP2, EPCAM and MAFB.

13. The bio-printed kidney tissue of claim 12, wherein the bio-printed kidney tissue comprises nephrons in which the proximal tubule and distal tubule segments express markers of maturation, including HNF4A.

14. The bio-printed kidney tissue of claim 12 or 13, wherein the bio-printed kidney tissue expresses each of the markers HNF4A, CUBN, LRP2, EPCAM and MAFB.

15. The bio-printed kidney tissue of any one of claims 1 - 14, wherein the tissue comprises from 5 to 100 nephrons / 10,000 cells printed.

16. The bio-printed kidney tissue of any one of claims 1 - 14, wherein the tissue has an even distribution of nephrons throughout the bio-printed layer.

17. The bio-printed kidney tissue of any one of claims 1 - 16, wherein the tissue has an even distribution of glomerular structures expressing MAFB throughout the bio-printed layer.

18. 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.

19. The method of claim 18, wherein the predetermined amount of bio-ink comprises between 10,000 cells/μl and 400,000 cells/μl.

20. The method of claim 18 or 19, wherein said plurality of cells comprises partly differentiated cells, renal progenitor cells, nephron progenitor cells, ureteric epithelial progenitor cells, intermediate mesoderm cells, metanephric mesenchyme cells, nephric duct cells, fully differentiated cells, patient-derived cells, cells from a reporter cell line, gene-edited cells, and/or diseased cells, healthy cells, or a combination of diseased and healthy cells.

21. The method of any one of claims 18 - 20, wherein the bio-printed kidney tissue comprises from 5 to 100 nephrons / 10,000 cells printed.

22. The method of any one of claims 18 - 21, wherein at the step of bio-printing the bio-ink is bio-printed onto a bio-compatible scaffold.

23. The method of any one of claims 18 - 22, wherein the step of inducing comprises contacting the bio-printed, predetermined amount of bio-ink with FGF-9.

24. The method of any one of claims 18 - 23, wherein the plurality of cells is contacted with a cell culture medium comprising CHIR before being bio-printed.

25. Bio-printed kidney tissue produced according to the method of any one of claims 18 – 24.

26. Bio-printed kidney tissue of any one of claims 1 – 17, or 25, for use in the treatment of kidney disease or renal failure in a subject in need thereof.

27. Use of bio-printed kidney tissue of any one of claims 1 – 17, or 25, in the manufacture of a medicament for the treatment of kidney disease in a subject in need thereof.