

CLAIMS

1. A kidney organoid comprising less than 50 nephrons.
- 5 2. The kidney organoid of claim 1, which comprises less than 25 nephrons.
3. The kidney organoid of claim 1, which comprises less than 15 nephrons.
4. The kidney organoid of claim 1, which comprises between 5 and 12 nephrons.
- 10 5. The kidney organoid according to any one of claims 1 to 4, which comprises cells expressing high levels of any one or more of PAX2, SIX1, LHX1, OSR1, WNT11, GATA3, PAX8, EYA1 and CITED1 and/or low levels of any one or more of PDGFRA, MEIS2, WT1 and/or C-RET.
- 15 6. The kidney organoid according to any one of claims 1 to 4, which comprises cells expressing high levels of PAX2, SIX1, LHX1, OSR1, WNT11 and GATA3.
- 20 7. The kidney organoid according to any one of claims 1 to 6, which comprises NPHS⁺ podocytes, LTL⁺ proximal segments, ECAD⁺ distal segments, ECAD⁺/GATA3⁺ collecting duct or a combination thereof.
- 25 8. The kidney organoid according to any one of claims 1 to 7, wherein the organoid is derived from stem cells selected from the group consisting of H9, hES3, iPSC GAPTrap *td-Tomato*, CRL1502.C32, CLR1502.3, hES3 *SOX17mCherry* or H9 GAPTrap *Luc2*.
- 30 9. The kidney organoid according to any one of claims 1 to 8, which comprises about 1×10^4 to 5×10^4 cells.
10. The kidney organoid according to any one of claims 1 to 8, which comprises about 1.5×10^4 to 2.5×10^4 cells.
- 35 11. The kidney organoid according to any one of claims 1 to 10, which has a diameter of about 250 to 500 μm .

12. The kidney organoid according to any one of claims 1 to 11, wherein the kidney organoid remains viable in swirler culture for at least three weeks.
- 5 13. The kidney organoid according to any one of claims 1 to 11, wherein the kidney organoid remains viable in culture for at least four weeks.
14. The kidney organoid according to any one of claims 1 to 13, wherein the organoids nephrons comprise collecting duct (GATA3+; ECAD+), early distal
10 tubule (GATA3-; LTL-; ECAD+), early proximal tubule (LTL+; ECAD-) and glomerulus (WT1+).
15. The kidney organoid according to any one of claims 1 to 14, wherein the organoid is produced by swirling a population of intermediate mesoderm (IM)
15 cells in a cell culture medium under conditions sufficient to promote kidney organoid development.
16. The kidney organoid of claim 15, wherein the cell culture medium comprises 200 ng/ml FGF9.
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17. The kidney organoid of claims 15 or 16, wherein the kidney organoid is produced by swirling between 0.5×10^6 and 1.5×10^6 IM cells/ml.
18. The kidney organoid of claims 15 or 16, wherein the kidney organoid is
25 produced by swirling 0.8×10^6 to 1.2×10^6 IM cells/ml.
19. A therapeutic composition which comprises a kidney organoid according to any one of claims 1 to 18 or an enzymatic digest thereof.
- 30 20. Method of treating kidney disease comprising, administering the composition of claim 19 to a subject in need thereof.
21. An *in-vitro* method of producing a kidney organoid, the method comprising, swirling a population of intermediate mesoderm (IM) cells in a cell culture
35 medium comprising FGF.

22. The method of claim 21, wherein the IM cells are swirled in culture for at least 5 days, wherein the first 24 hours comprise swirling cells in a cell culture medium comprising FGF, heparin, CHIR and ROCK inhibitor and the next four days comprise culturing cells in a cell culture medium comprising FGF, heparin and CHIR.
23. The method of claim 22, wherein the remaining days in culture comprises culturing cells in a cell culture medium comprising PVA and MC.
24. The method of claim 22, wherein the cell culture mediums comprise from 100 to 300 ng/ml FGF9.
25. The method according to any one of claims 21 to 24, wherein the first 24 hours comprise swirling cells in a cell culture medium comprising FGF, 0.5 to 1.5 µg/ml heparin, 0.5 to 1.5 µM CHIR and 9 to 11 µM ROCK inhibitor.
26. The method according to any one of claims 21 to 25, wherein the next four days comprise swirling cells in a cell culture medium comprising FGF, 0.5 to 1.5 µg/ml heparin and 0.5 to 1.5 µM CHIR.
27. The method according to any one of claims 21 to 26, wherein the next four days comprise culturing cells in a cell culture medium comprising FGF9, heparin, CHIR, MVA and PVC.
28. The method of claim 27, wherein the cell culture medium comprising FGF comprises 0.05 to 0.2% MVA and 0.05 to 0.2% PVC.
29. The method according to any one of claims 21 to 28, wherein the remaining days in culture comprises culturing cells in a cell culture medium comprising 0.05 to 0.2% PVA and 0.05 to 0.2% MC, without FGF, heparin CHIR or ROCK inhibitor.
30. The method according to any one of claims 21 to 29, wherein the IM cells are swirled between 30 and 90 rpm.

31. The method according to any one of claims 21 to 30, wherein the IM cells are swirled for 18 to 24 days.
- 5 32. The method according to any one of claims 21 to 31, wherein the IM cells are produced by culturing a population of stem cells for at least seven days, wherein the first 4 to 5 days comprise culturing stem cells in a cell culture medium comprising at least 6 μM of a Wnt/ β -catenin agonist and the remaining days in culture comprises culturing cells in a cell culture medium comprising FGF and at least 0.5 μM of a Wnt/ β -catenin agonist.
- 10 33. The method of claim 32, wherein the Wnt/ β -catenin agonist is CHIR, and the FGF is FGF9.
- 15 34. The method of claims 32 or 33, wherein the cell culture medium comprising FGF comprises between 100 to 300 ng/ml of FGF9.
35. The method according to any one of claims 33 to 34, wherein the cell culture medium comprising FGF comprises from 0.5 to 1.5 μM CHIR.
- 20 36. The method according to any one of claims 32 to 35, wherein the cell culture medium comprising FGF further comprises 0.5 to 1.5 $\mu\text{g/ml}$ heparin.
- 25 37. The method according to any one of claims 21 to 36, wherein the IM cells are dissociated with EDTA or trypsin and passed through a mesh screen before swirling.
- 30 38. The method according to any one of claims 32 to 37, wherein the stem cells are pluripotent stem cells, embryonic stem cells or induced pluripotent stem (iPS) cells.
39. The method according to any one of claims 21 to 38, which comprises swirling an IM cell population which comprises from 0.5×10^6 IM cells/ml to 1.5×10^6 IM cells/ml.
- 35 40. A kidney organoid produced by the method according to any one of claims 21 to 39.

41. Method of screening a candidate compound for nephrotoxicity, the method comprising contacting a kidney organoid according to any one of claims 1 to 18 or 40 with a candidate compound to determine whether or not the candidate compound is nephrotoxic.
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42. The method of claim 41, wherein the candidate compound is a small molecule.
43. The kidney organoid defined by any one of claims 1 to 18 or 40 or the composition according to claim 19 when used for producing a kidney, or kidney cells or tissues.
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44. An *in-vitro* method of producing intermediate mesoderm (IM) cells, the method comprising culturing a population of posterior primitive streak (PPS) cells for 2 to 5 days in a cell culture medium comprising FGF and less than 4 μ M of a Wnt/ β -catenin agonist.
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45. An *in-vitro* method of producing intermediate mesoderm (IM) cells, the method comprising, culturing a population of stem cells for at least seven days, wherein the first 4 to 5 days comprise culturing stem cells in a cell culture medium comprising at least 6 μ M of a Wnt/ β -catenin agonist and the remaining days in culture comprises culturing cells in a cell culture medium comprising FGF9 and at least 0.5 μ M of a Wnt/ β -catenin agonist.
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46. The method of claim 45, wherein the culture medium comprising FGF comprises from 0.5 to 3 μ M of a Wnt/ β -catenin agonist.
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47. The method of claim 45, wherein the culture medium comprising from 0.8 to 1.2 μ M of a Wnt/ β -catenin agonist.
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48. The method according to any one of claims 45 to 47, wherein the first 4 days comprise culturing stem cells in a cell culture medium comprising at least 6 μ M of a Wnt/ β -catenin agonist.

49. The method according to any one of claims 45 to 47, wherein the first 4 days comprise culturing stem cells in a cell culture medium comprising 7 μ M of a Wnt/ β -catenin agonist.
- 5 50. The method according to any one of claims 44 to 49, wherein the cell culture medium comprising FGF comprises from 100 to 300 ng/ml FGF9.
51. The method according to any one of claims 44 to 50, wherein the cell culture medium comprising FGF further comprises heparin.
- 10 52. The method of claim 51, wherein the cell culture medium comprises from 0.5 to 2 μ g/ml heparin.
- 15 53. The method according to any one of claims 45 to 52, wherein the stem cells are pluripotent stem cells, embryonic stem cells or induced pluripotent stem (iPS) cells.
54. The method of claim 20, wherein the composition is administered intravenously.
- 20 55. The method of claim 20, wherein the composition is administered via kidney artery injection, kidney parenchymal injection, implantation or subcapsular transplantation.
56. Method of bio-printing a kidney comprising:
- 25 - preparing a bioink from an organoid defined by any one of claims 1 to 18 or 40;
- bio-printing a kidney.
57. The organoid defined by any one of claims 1 to 18 or 40 or the composition according to claim 19 when used for producing a kidney, or kidney cells or tissues.
- 30 58. A method of generating nephron cell types for cellular therapy, the method comprising producing a kidney organoid using a method defined in any one of claims 21 to 39 or IM cells using a method defined by claims 44 to 53.
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