In one aspect, the present disclosure refers to an *in-vitro* method of producing a kidney organoid, the method comprising, swirling a population of intermediate mesoderm (IM) cells in a cell culture medium comprising fibroblast growth factor (FGF), wherein the kidney organoid has a diameter less than 500 µm and comprises NPHS1+ podocytes, LTL+ proximal segments, ECAD+ distal segments and ECAD+/GATA3+ collecting duct.

In another aspect, the present disclosure refers to a kidney organoid comprising less than 50 nephrons produced by the method disclosed herein, wherein the organoid has a diameter less than 500 µm and comprises NPHS1+ podocytes, LTL+ proximal segments, ECAD+ distal segments and ECAD+/GATA3+ collecting duct.

In yet another aspect, the present disclosure refers to a kidney organoid comprising less than 50 nephrons, wherein the organoid has a diameter less than 500 µm and comprises NPHS1+ podocytes, LTL+ proximal segments, ECAD+ distal segments and ECAD+/GATA3+ collecting duct~~kidney organoid comprising less than 50 nephrons, which comprises cells expressing high levels of any one or more of PAX2, SIX1, LHX1, OSR1, WNT11, GATA3, PAX8, EYA1 and CITED1 relative to kidney organoids cultured without swirling~~.

In yet another aspect, the present disclosure refers to use of a composition which comprises a kidney organoid disclosed herein or a cellular composition thereof that is prepared by dissociating the kidney organoid with a protease either alone or in combination with Ethylenediaminetetraacetic acid (EDTA) or with a collagenase~~trypsin or collagenase~~, in the manufacture of a medicament for the treatment of kidney disease.

The present inventors have surprisingly identified kidney organoids having a simplified three dimensional structure. Such organoids are advantageous as they are

CLAIMS

1. An *in-vitro* method of producing a kidney organoid, the method comprising, swirling a population of intermediate mesoderm (IM) cells in a cell culture medium comprising fibroblast growth factor (FGF) , wherein the kidney organoid has a diameter less than 500 µm and comprises NPHS1+ podocytes, LTL+ proximal segments, ECAD+ distal segments and ECAD+/GATA3+ collecting duct.

2. The method of claim 1, 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 rho kinase (ROCK) inhibitor and the next four days comprise culturing cells in a cell culture medium comprising FGF, heparin and CHIR.

3. The method of claim 2, wherein the remaining days in culture comprises culturing cells in a cell culture medium comprising poly vinyl alcohol (PVA) and methyl cellulose (MC).

4. The method of claim 2, wherein the cell culture mediums comprise from 100 to 300 ng/ml FGF.

5. The method according to any one of claims 2 to 4, wherein the first 24 hours comprise swirling cells in a cell culture medium comprising 100 to 300 ng/ml FGF, 0.5 to 1.5 µg/ml heparin, 0.5 to 1.5 µM CHIR and 9 to 11 µM ROCK inhibitor.

6. The method according to any one of claims 2 to 5, wherein the next four days comprise swirling cells in a cell culture medium comprising 100 to 300 ng/ml FGF, 0.5 to 1.5 µg/ml heparin and 0.5 to 1.5 µM CHIR.

7. The method according to any one of claims 2 to 6, wherein the next four days comprise culturing cells in a cell culture medium further comprising PVA and MC.

8. The method according to any one of claims 1 to 7, wherein the IM cells are swirled between 30 and 90 rpm.

9. The method according to any one of claims 1 to 8, wherein the IM cells are swirled for 18 to 24 days.

10. The method according to any one of claims 1 to 9, 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.

11. The method of claim 10, wherein the Wnt/β-catenin agonist is CHIR, and the FGF is FGF9.

12. The method according to claim 10 or 11, wherein the cell culture medium comprising FGF further comprises 0.5 to 1.5 µg/ml heparin.

13. The method according to any one of claims 1 to 12, wherein the IM cells are dissociated with Ethylenediaminetetraacetic acid (EDTA) or trypsin and passed through a mesh screen before swirling.

14. The method according to any one of claims 10 to 13, wherein the stem cells are pluripotent stem cells, embryonic stem cells or induced pluripotent stem (iPS) cells.

15. The method according to any one of claims 1 to 14, which comprises swirling an IM cell population which comprises from 0.5 x 10^6 IM cells/ml to 1.5 x 10^6 IM cells/ml.

~~16. A kidney organoid produced by the method according to any one of claims 1 to 15.~~

16. A kidney organoid comprising less than 50 nephrons, wherein the organoid has a diameter less than 500 µm and comprises NPHS1+ podocytes, LTL+ proximal segments, ECAD+ distal segments and ECAD+/GATA3+ collecting duct.

17. The kidney organoid according to claim 16~~A kidney organoid comprising less than 50 nephrons~~, which comprises cells expressing high levels of any one or more of PAX2, SIX1, LHX1, OSR1, WNT11, GATA3, PAX8, EYA1 and CITED1 relative to kidney organoids cultured without swirling.

18. The kidney organoid according to ~~of~~ claim ~~17~~16 or 17, which comprises cells expressing low levels of any one or more of PDGFRA, MEIS2, WT1 and/or C-RET relative to kidney organoids cultured without swirling.

19. The kidney organoid according to any one of claims 16 to 18, which comprises at least 25% mature nephron and at least 15% stroma.

~~19. The kidney organoid according to claim 17 or 18, which comprises NPHS+ podocytes, LTL+ proximal segments, ECAD+ distal segments, ECAD+/GATA3+ collecting duct or a combination thereof.~~

20. The kidney organoid according to any one of claims 17 to 19, which comprises 1 x 10^4 to 5 x 10^4 cells.

21. The kidney organoid according to any one of claims 17 to 20, which has a diameter of 250 to 500 µm.

22. The kidney organoid according to any one of claims 17 to 21, wherein the kidney organoid remains viable in swirler culture for at least three weeks.

23. The kidney organoid according to any one of claims 17 to 22, wherein the nephrons comprise GATA3+/ECAD+ collecting duct, GATA3-/LTL-/ECAD+ early distal tuble , LTL+/ECAD- early proximal tuble and WT1+ glomerulus.

24. Use of a composition which comprises a kidney organoid according to any one of claims 17 to 23 or a cellular composition thereof that is prepared by dissociating the kidney organoid with a protease either alone or in combination with Ethylenediaminetetraacetic acid (EDTA) or with a collagenase~~trypsin or collagenase~~, in the manufacture of a medicament for the treatment of kidney disease.