Accordingly, one aspect of the invention provides a method of producing nephron progenitor cells and ureteric epithelial progenitor cells including the step of contacting intermediate mesoderm (IM) cells with: fibroblast growth factor 9 (FGF9) and/or fibroblast growth factor 20 (FGF20); and optionally, one or more agents selected from the group consisting of: bone morphogenic protein 7 (BMP7); heparin; a Wnt agonist; retinoic acid (RA), analog or agonist; and an RA antagonist; to thereby produce nephron progenitor cells and ureteric, epithelial progenitor cells from the IM cells.

In one example, the IM cells are derived or differentiated from posterior primitive streak cells.

In one example, the posterior primitive streak cells are derived or differentiated front human pluripotent stem cells (hPSCs). Non-limiting examples of hPSCs include human embryonic stem cells (hESCs) and induced human pluripotent stem cells (iPSCs),

In one aspect, there is disclosed a method that includes the sequential steps of

(i) contacting hPSCs with one or more agents that facilitate differentiation of the hPSCs into posterior primitive streak cells;
(ii) contacting the posterior primitive streak cells with one or more agents that facilitate differentiation of the posterior primitive streak, cells into IM cells; and
(iii) contacting IM cells with FGF9 alone or in combination with one or more of: BMP7 RA; an RA antagonist; a Wnt agonist; and/or FGF2O; and heparin; to thereby produce nephron progenitor cells and ureteric epithelial progenitor cells from the IM cells.

The one or more agents at step (ii) preferably include FGF9. In one particular example, FGF9 is present for at least part of, or entirely throughout, both steps (ii) and (iii). In another example, a Wnt agonist such as CHIR99021 is present during step (i).

In one example, the method further includes the step of identifying viable nephron progenitor cells and/or ureteric epithelial progenitor cells.

In certain examples, identification of viable nephron progenitor cells and/or ureteric epithelial progenitor cells includes measurement or detection of co-

expression of a plurality of nucleic acids and/or protein as markers for the viable nephron and/or ureteric epithelial progenitor cells.

In another aspect, the invention provides isolated, enriched or purified nephron and/or ureteric epithelial progenitor cells produced according to the method as disclosed herein

In yet another aspect, the invention provides a method of producing a kidney, or kidney cells or tissues, said method including the step of differentiating kidney, or kidney: cells or tissues from the nephron progenitor cells and/or ureteric epithelial progenitor cells as disclosed herein to thereby produce the kidney, or kidney cells or tissues.

In some examples, the nephron progenitor cells and/or ureteric epithelial progenitor cells may be used as a source for bioprinting or bio-engineering whole kidneys and kidney tissue for kidney transplant or treating chronic kidney disease.

In other examples, the nephron progenitor cells and/or ureteric epithelial progenitor cells may be used for the recellularisation of whole organ decellularised kidney to thereby create a reconstituted or replacement kidney.

In other examples, the nephron progenitor cells and/or ureteric epithelial progenitor cells may be used as a source for cellular therapy of kidney diseases and conditions.

In a further aspect, the invention provides a method of determining the nephrotoxicity of one or a. plurality of compounds, said method including the step of contacting the one or plurality of compounds with the isolated or purified nephron progenitor cells and/or ureteric epithelial progenitor cells of the aforementioned aspect, or kidney cells or tissues differentiated or otherwise obtained therefrom, to thereby determine whether or not the one or plurality of compounds is nephrotoxic.

In one example, this aspect provides bioprinting of the nephron progenitors and/or ureteric epithelial progenitors into kidney organoids for nephrotoxicity screening is disclosed.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Sequential differentiation of posterior primitive streak and intermediate mesoderm from human embryonic stem cells. **a,** Schematic of developmental stages from inner cell mass to renal lineages. Genes shown in each stage represent specific markers of that stage. b, FACS analysis (GFP and forward scatter (FSC)) showing

CLAIMS

1. A method of producing nephron progenitor cells and ureteric epithelial progenitor cells including the step of contacting intermediate mesoderm (IM) cells with: fibroblast growth factor 9 (FGF9) and/or fibroblast growth factor 20 (FGF20); and optionally, one or more selected from the group consisting of: bone morphogenic protein 7 (BMP7); heparin; a Wnt agonist; retinoic acid (RA), analog or agonist; and an RA antagonist; to thereby produce nephron progenitor cells and ureteric epithelial progenitor cells from the IM cells.

2. The method of Claim 1, wherein FGF9 and/or FGF 20 is at a concentration selected from the group consisting of: in the range of about 20 ng to 1 μg/mL; in the range of about 50-500 ng/mL; in the range of about 100-300 ng/mL; and about 200 ng/mL.

3. The method of Claim 1 or Claim 2, wherein BMP7 is present at a concentration selected from the group consisting of: in the range of about 25 to 75 ng/mL; in the range of about 35 to 60 ng/mL; in the range of about 45 to 55 ng/mL; and about 50 ng/mL.

4. The method of any preceding claim, wherein RA, analog or agonist, increases the relative production of ureteric epithelial progenitor cells from the IM cells.

5. The method of any preceding claim, wherein RA, analog or agonist, is present at a concentration selected from the group consisting of: in the range of about 10 pM to 1 μM; in the range of about 30 pM to 0.5 μM; in the range of about 50 pM to 0.2 μM; and about 0.1 μM.

6. The method of any preceding claim, wherein the RA antagonist decreases the relative production of ureteric progenitor cells from the IM cells.

7. The method of any preceding claim, wherein the RA antagonist is present at a concentration selected from the group consisting of: in the range of about 0.50 pM to 10 μM; in the range of about 0.01 μM to 5 μM; in the range of about 0.1 μM to 5 μM; and about 1 μM.

8. The method of any preceding claim, wherein the Wnt agonist increases the relative production of nephron progenitor progenitor cells from the IM cells.

9. The method of any preceding claim, wherein the Wnt agonist is present at a concentration selected from the group consisting of: in the range of about 0.1 μM to 10 μM; in the range of about 0.2 μM to 5 μM; and in the range of about 1-2 μM.

10. The method of any preceding claim, wherein heparin is present at a concentration in the range of about 0.1-10 μg/mL.

11. The method of any preceding claim, wherein the intermediate mesoderm (IM) cells are contacted with FGF9 alone or in combination with one or more of BMP7, RA, the RA antagonist, the Wnt agonist, FGF20 and/or heparin for about 72-360 hrs.

12. The method of any preceding claim, wherein the nephron progenitor cells and ureteric epithelial progenitor cells are produced synchronously or simultaneously from the IM cells.

13. The method of any preceding claim, which includes contacting posterior primitive streak cells with one or more agents that facilitate differentiation of the posterior primitive streak cells into said IM cells.

14. The method of Claim 13, which includes contacting human pluripotent stem cells (hPSCs) with one or more agents that facilitate differentiation of the hPSCs into said posterior primitive streak cells.

15. The method of Claim 14, wherein the hPSCs are human embryonic stem cells or induced human pluripotent stem cells.

16. The method of any preceding claim that includes the sequential steps of:

(a) contacting human pluripotent stem (hPCS) cells with one or more agents that facilitate differentiation of the hPSC cells into posterior primitive streak cells;

(b) contacting posterior primitive streak cells with one or more agents that facilitate differentiation of the posterior primitive streak cells into IM cells; and

(c) contacting IM cells with FGF9 in combination with one or more of BMP7; RA; an RA antagonist; a Wnt agonist; FGF20; and/or heparin to thereby produce nephron progenitor cells and ureteric epithelial progenitor cells from the IM cells.

17. The method of any one of Claims 13 to 16, wherein the one or more agents contacted with the posterior primitive streak cells include fibroblast growth factor 9 (FGF9) alone or in combination with FGF2 and/or FGF20.

18. The method of Claim 17, wherein FGF9, FGF2 and/or FGF20 are at a concentration of about 100 to 400 ng/mL.

19. The method of any one of Claims 14 to 18, wherein the one or more agents contacted with the hPSCs include BMP4, Activin A and/or a Wnt agonist.

20. The method of Claim 19, wherein (i) BMP4 is at a concentration of about 5-40 ng/mL; (ii) Activin A is at a concentration of about 3-40 ng/mL; and/or the Wnt agonist is at a concentration in the range of about 0.5 to 50 μM.

21. The method of Claim 16 that includes the sequential steps of

(a) contacting human pluripotent stem (hPCS) cells with a Wnt agonist to facilitate differentiation of the hPSC cells into posterior primitive streak cells;

(b) contacting the posterior primitive streak cells with FGF9, alone or together with an RA antagonist, to facilitate differentiation of the posterior primitive streak cells into IM cells; and

(c) contacting the IM cells with FGF9 alone or in combination with one or more of BMP7; RA; an RA antagonist: a Wnt agonist; FGF20; and/or heparin to thereby produce nephron progenitor cells and ureteric epithelial progenitor cells from the IM cells.

22. The method of any preceding claim, further including the step of identifying viable nephron progenitor cells and/or ureteric epithelial progenitor cells.

23. The method of Claim 22, wherein identification of viable nephron progenitor cells and/or ureteric epithelial progenitor cells includes measurement or detection of co-expression of a plurality of nucleic acids and/or proteins as markers that identify or distinguish viable nephron and/or ureteric epithelial progenitor cells.

24. Isolated, enriched or purified nephron progenitor cells and/or ureteric epithelial progenitor cells produced according to the method of any one of Claims 1 to 23.

25. A method of producing a kidney, or kidney cells or tissues, said method including the step of differentiating the kidney, or the kidney cells or tissues from the isolated or purified nephron and/or ureteric epithelial progenitor cells of Claim 24, to thereby produce the kidney, or kidney cells or tissues.

26. The isolated, enriched or purified nephron progenitor cells and/or ureteric epithelial progenitor cells of Claim 24 for use in producing a kidney, or kidney cells or tissues.

27. The nephron progenitor cells and/or ureteric epithelial progenitor cells of claim 24 for use in (i) bioprinting or bio-engineering whole kidneys and kidney tissue for kidney transplant or treating chronic kidney disease; (ii) the recellularisation of whole organ decellularised kidney to thereby create a reconstituted or replacement kidney; or (iii) cellular therapy of kidney diseases and conditions.

28. A method of determining the nephrotoxicity of one or a plurality of compounds, said method including the step of contacting the one or plurality of compounds with the isolated or purified nephron progenitor cells and/or ureteric epithelial progenitor cells of Claim 26, or kidney cells or tissues differentiated or otherwise obtained therefrom, to thereby determine whether or not the one or plurality of compounds is nephrotoxic.

29. The method of Claim 28, wherein the method is performed by contacting the one or plurality of compounds with a renal organoid.

30. The method of Claim 1, that includes the step of contacting IM cells with:

(a) fibroblast growth factor 9 (FGF9) and/or fibroblast growth factor 20 (FGF20); and

(b) one or more selected from the group consisting of: heparin; a Wnt agonist; retinoic acid (RA), analog or agonist; and an RA antagonist.