

CLAIMS

1. A method for generating thymic epithelial progenitor (TEP) cells *in vitro*, the method comprising:

culturing a population of cells in a first medium comprising an activator of bone morphogenetic protein (BMP) signaling, an activator of fibroblast growth factor (FGF) signaling, and an inhibitor of transforming growth factor- β (TGF- β) signaling, and

further culturing the population of cells to induce further maturation of the TEP cells *in vitro*, wherein the further culturing comprises culturing the population of cells in a second medium comprising keratinocyte growth factor (KGF), heparin, and hydrocortisone.
2. The method of claim 1, wherein the second medium further comprises a triiodo-L-thyronine (T3) supplement.
3. The method of claim 1 or 2, wherein the second medium further comprises an insulin-transferrin-selenium (ITS) supplement.
4. The method of any one of claims 1-3, wherein the first and/or second medium further comprises a B27 supplement.
5. The method of any one of claims 1-4, wherein the population of cells comprises one or more of definitive endodermal (DE) cells, anterior foregut endodermal (AFE) cells, ventral pharyngeal endodermal (VPE) cells, and TEP cells.
6. The method of any one of claims 1-4, wherein the further culturing the population of cells to induce further maturation of the TEP cells *in vitro* comprises further culturing the population of cells for up to 14 days.
7. The method of any one of claims 1-6, wherein the further culturing the population of cells to induce further maturation of the TEP cells *in vitro* comprises transferring the population of cells to an extracellular matrix-based medium such as Matrigel.
8. The method of any one of claims 1-6, wherein the first and/or second medium is a liquid medium and the culture conditions comprise suspension culture.
9. The method of claim 8, wherein the first and/or second medium is a minimum essential medium or Dulbecco's minimum essential medium (DMEM).

10. The method of any one of claims 1-9, further comprising transplanting the TEP cells to a subject, wherein the further culturing the population of cells to induce further maturation of the TEP cells *in vitro* gives rise to subpopulations of thymic epithelial cells (TECs) after transplantation *in vivo*.

11. The method of claim 10, wherein the subpopulations of TECs comprise one or more of cortical TEC (cTEC) lineage cells, bipotent TEP cells, committed medullary TEC (mTEC) progenitor cells, immature mTECs, mature mTECs, post-AIRE mTECs, tuft cells, neuroendocrine cells, ionocytes, ciliated cells, myelin expressing cells, and/or myoid cells.

12. The method of any one of claims 1-11, further comprising transferring the population of cells to an air-liquid interface culture system before transplanting.

13. The method of any one of claims 1-12, further comprising reaggregating the cells to form a reaggregate before transplanting.

14. The method of any one of claims 1-13, further comprising reducing or eliminating non-epithelial cells from the culture of differentiated TEP cells.

15. The method of claim 14, comprising enriching for EPCAM+ TEP cells.

16. The method of any one of claims 1-15, comprising adjusting culture conditions or combining cell types in culture to recapitulate a thymic microenvironment.

17. The method of claim 16, wherein recapitulating the thymic microenvironment comprises culturing TEP cells under conditions sufficient to support survival of lymphatic endothelium cells, vascular endothelium cells, immune cells, mesenchymal cells, pericytes, red blood cells, or combinations thereof.

18. The method of claim 16, wherein recapitulating the thymic microenvironment comprises culturing TEP cells under conditions sufficient to support differentiation of TECs and subpopulations thereof.

19. The method of claim 18, wherein the TECs and subpopulations thereof comprise cTEC lineage cells, bipotent TEP cells, committed mTEC progenitor cells, immature mTECs, mature mTECs, post-AIRE mTECs, tuft cells, neuroendocrine cells, ionocytes, ciliated cells, myelin expressing cells, and/or myoid cells.

20. The method of any one of claims 1-19, wherein the method comprises culturing definitive endodermal (DE) cells in the first medium comprising an activator of retinoic acid receptor, an activator of BMP signaling, an activator of FGF signaling, and an inhibitor of TGF- β signaling.
21. The method of claim 20, wherein the DE cells are differentiated in an initial cell culture medium comprising an inhibitor of BMP signaling in advance of the first medium comprising the activator of BMP signaling.
22. The method of claim 20 or 21, wherein an inhibitor of Wnt signaling is introduced into the first medium.
23. The method of any one of claims 20-22, wherein the method comprises culturing anterior foregut endodermal (AFE) cells produced by said culturing of the DE cells, wherein said culturing of the AFE cells is in the first medium comprising an activator of BMP signaling, an activator of FGF signaling, and an inhibitor of TGF- β signaling.
24. The method of claim 23, wherein the method comprises culturing ventral pharyngeal endodermal (VPE) cells produced by said culturing of the AFE cells, wherein said culturing of the VPE cells is in the first medium comprising an activator of BMP signaling, an activator of FGF signaling, and an inhibitor of TGF- β signaling.
25. The method of claim 23 or 24, wherein the first medium is not supplemented with an activator of retinoic acid receptor signaling.
26. The method of any one of claims 1-25, wherein the starting cells are obtained from pluripotent stem (PS) cells.
27. The method of claim 26, wherein the PS cells are embryonic stem cells, embryonic germ cells, or induced pluripotent stem (iPS) cells.
28. The method of claim 26 or 27, wherein the PS cells are primate pluripotent stem cells (pPS) cells.
29. The method of any one of claims 26-28, wherein the PS cells are human pluripotent stem (hPS) cells.
30. A method for generating TEP cells *in vitro*, the method comprising culturing a

population of cells comprising anterior foregut endodermal (AFE) cells in a first cell culture medium comprising an activator of BMP signaling, an activator of FGF signaling, and an inhibitor of TGF- β signaling to produce TEP cells.

31. The method of claim 30, wherein the first cell culture medium is substantially free of at least one of: an activator of retinoic acid receptor signaling, an activator of Wnt signaling, and an inhibitor of hedgehog signaling.

32. A method for generating TEP cells *in vitro*, the method comprising culturing a population of cells comprising definitive endodermal (DE) cells in a first cell culture medium comprising an activator of BMP signaling, an activator of retinoic acid receptor signaling, an activator of FGF signaling, an inhibitor of TGF- β signaling, and an inhibitor of Wnt signaling to produce a population of cells comprising AFE cells, and culturing the population of AFE cells in a cell culture medium comprising an activator of BMP signaling, an activator of FGF signaling, an inhibitor of TGF- β signaling, and substantially free of an inhibitor of Wnt signaling to produce TEP cells.

33. The method of any one of claims 30-32, wherein the first cell culture medium further comprises an insulin-transferrin-selenium (ITS) supplement.

34. The method of any one of claims 30-33, wherein the first cell culture medium further comprises B27.

35. The method of any one of claims 30-34, wherein the first cell culture medium comprises a minimum essential medium or Dulbecco's minimum essential medium (DMEM).

36. The method of any one of claims 30-35, further comprising culturing the TEP cells in a second cell culture medium comprising KGF and T3 under conditions sufficient to cause further differentiation of the TEP cells to generate a population of mature TEP cells.

37. A composition comprising a differentiated population of TEP cells produced according to the method of any one of claims 1-36.

38. A composition comprising reaggregated thymic epithelial progenitor (TEP) cells differentiated from PS cells, wherein the composition further comprises one or more cell type selected from lymphatic endothelium cells, vascular endothelium cells, immune cells, mesenchymal cells, pericytes, red blood cells, or combinations thereof.

39. A composition comprising TECs differentiated from PS cells, wherein the composition further comprises one or more of cTEC lineage cells, bipotent TEP cells, committed mTEC progenitor cells, immature mTECs, mature mTECs, post-AIRE mTECs, tuft cells, neuroendocrine cells, ionocytes, ciliated cells, myelin expressing cells, and/or myoid cells.

40. A composition comprising reaggregated thymic epithelial cells (TECs) differentiated from PS cells, wherein the composition further comprises one or more cell type selected from lymphatic endothelium cells, vascular endothelium cells, immune cells, mesenchymal cells, pericytes, red blood cells, or combinations thereof.

41. The composition of claim 40, wherein the reaggregated TECs comprise subpopulations including one or more of cTEC lineage cells, bipotent TEP cells, committed mTEC progenitor cells, immature mTECs, mature mTECs, post-AIRE mTECs, tuft cells, neuroendocrine cells, ionocytes, ciliated cells, myelin expressing cells, and/or myoid cells.

42. The method of any one of claims 1-29, 30-31, or 32-36, further comprising directing development of one or more subpopulation of thymic epithelial cells by introducing to the medium one or more of:

- a. factors associated with WNT signaling;
- b. factors associated with BMP signaling;
- c. factors associated with TGF beta signaling;
- d. factors associated with IGF signaling;
- e. factors associated with FGF signaling;
- f. factors associated with NOTCH signaling;
- g. TNF receptors;
- h. factors associated with p53 signaling; and/or
- i. Toll-like receptors.

43. The method of claim 42, wherein the introducing to the medium comprises introducing a soluble form of the one or more factors and/or introducing a cell that expresses the one or more factors.

44. The method of any one of claims 16-18, wherein the thymic microenvironment is characterized by presence of cells expressing one or more of:

- a. factors associated with WNT signaling;
- b. factors associated with BMP signaling;

- c. factors associated with TGF beta signaling;
- d. factors associated with IGF signaling;
- e. factors associated with FGF signaling;
- f. factors associated with NOTCH signaling;
- g. TNF receptors;
- h. factors associated with p53 signaling; and/or
- i. Toll-like receptors.

45. The method of any one of claims 42-44, wherein:

- a. factors associated with WNT signaling comprise WNT5A, WNT6, ROR1, ROR2, RYK, FRZB, RSPO1, RSPO3, SFRP2, and/or SFRP5;
- b. factors associated with the BMP signaling comprise BMP4, BMP5, and/or FST;
- c. factors associated with TGF beta signaling comprise TGFB1, TGFB2, CXCL12, and/or CCL21;
- d. factors associated with IGF signaling comprise IGF1R;
- e. factors associated with FGF signaling comprise FGFR2 and/or FGF7/KGF;
- f. factors associated with NOTCH signaling comprise NOTCH1, NOTCH2, NOTCH3, HES1, HES6, DLL4, JAG2, JAG1, HES2, HES4, HEY1, NRARP, DLK1, and/or DLK2;
- g. TNF receptors comprise RANK/TNFRSF11A, CD40, LTBR, TNFRSF4, TNFRSF9, LTB, and/or CD70;
- h. factors associated with p53 signaling comprise PERP, SFN, CTSD, CDKN2A, and/or CDKN2B; and
- i. Toll-like receptors comprise TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, and/or TLR10.