CLAIMS

1. A method of differentiating pluripotent stem cells to thymic cells, comprising:

a) differentiating the pluripotent stem cells into definitive endoderm (DE) cells;

b) culturing the DE cells and differentiating the DE cells into anterior foregut endoderm (AFE) cells by contacting or incubating the DE cells with a BMP inhibitor, a TGFβ inhibitor, an FGF, an ascorbic acid or a combination thereof;

c) culturing the AFE cells and differentiating the anterior foregut cells into ventral pharyngeal endoderm (VPE) cells comprising:

i) contacting or incubating the AFE cells in a first VPE medium comprising ascorbic acid, a retinoic acid, an FGF, a TGFβ inhibitor or a combination thereof; and

ii) contacting or incubating the AFE cells in a second VPE medium comprising a Noggin, a WNT activator, an FGF, a retinoic acid, an ascorbic acid, or a combination thereof; and

d) culturing the VPE cells and differentiating the VPE cells into thymic cells, by contacting or incubating the VPE cells with an ascorbic acid, an FGF, a BMP, a WNT activator or a combination thereof;

wherein the thymic cells are thymic epithelial progenitor cells (TEPs) and/or thymic epithelial cells (TECs).

2. The method of claim 1, further comprising encapsulating the pluripotent stem cells in a polymer differentiating the pluripotent stem cells into definitive endoderm (DE) cells.

3. The method of claim 1, wherein the TEPs are further differentiated into thymic epithelial cells (TECs) by contacting or incubating the TEPs with an Interleukin, a WNT activator, a RANKL, an FGF, a BMP, an ascorbic acid, or a combination thereof.

4. The method of claim 1, wherein the differentiation of pluripotent stem cells to DE cells comprises:

a) contacting or culturing the pluripotent stem cells in a first growth medium, said first growth medium comprising Activin A, PI-103, CHIR99021 or a combination thereof; and

b) culturing the pluripotent stem cells in a second growth medium to generate definitive endoderm cells, wherein the second growth medium comprises: Activin A, a BMP inhibitor, PI-103, CHIR99021 or a combination thereof.

5. The method of claim 1, wherein:
(i) the first VPE media further comprises a WNT inhibitor; and/or
(ii) the second VPE media further comprises a BMP inhibitor, an SHH inhibitor, or a combination thereof .

6. The method of claim 2, wherein the polymer comprises a biogenic polymer, a synthetic polymer, a composite polymer, a hydrogel, or a cross-linked polymer, optionally whereinthe polymer is alginate.

7. The method of claim 1, wherein:
(i) the BMP inhibitor is LDN193189;
(ii) the TGFβ inhibitor is SB431542;
(iii) the FGF is FGF8b, FGF7, FGF10, FGF1, bFGF or a combination thereof;
(iv) the WNT activator is CHIR99021; and/or
(v) the BMP is BMP2, BMP4 or a combination thereof.

8. The method of ~~any one of claims 1-13~~claim 3, wherein:
(i) the interleukin is IL22;
(ii) the WNT inhibitor is IWR-1; and/or
(iii) the BMP is BMP2, BMP4 or a combination thereof.

9. The method of claim 5, wherein the SHH inhibitor is SANT-1.

10. The method of claim 1, wherein the pluripotent stem cells, the DE cells, the AFE cells, the VPE cells or thymic cells are cultured in 3D culture or cultured as aggregates in suspension .

11. The method of any one of claims 1-10, wherein the method is performed for from about 15 days to 30 days, optionally, wherein the method is performed for from about 18 days to 25 days.

12. The method of claim 1, wherein:

(i) the pluripotent stem cells are differentiated into definitive endoderm cells for about 5 days;
(ii) the DE cells are differentiated into AFE cells for from about 2 days to 3 days;
(iii) the AFE cells are cultured in the first VPE media for about 2 to 4 days;
(iv) the AFE cells are cultured in the second VPE media for about 2 to 3 days; and or
(v) the VPE cells are differentiated to thymic cells for about 3 to 6 days, optionally wherein the TEPs are differentiated to TECs for about 4 days .

13. The method of claim 2, wherein the polymer is removed from the TEPs or wherein the polymer is removed from the TECs .

14. A population of thymic cells prepared according to the method of claim 1.

15. A pharmaceutical composition comprising a population of thymic cells of claim 14 and at least one excipient.

16. A composition comprising:

a) a population of thymic cells, wherein the population of thymic cells comprises one or more of an iTEC, mTECs, Corneocyte-like mTEC, cTEC-high, cTEC- low, mTEC-low cell; and

b) a thymic support system (TSS).

17. The composition of claim 16, wherein the population of thymic cells is prepared by differentiation of iPS cells to thymic cells.

18. The composition of claim 17, wherein the TSS comprises a polymer, optionally wherein the polymer is a biogenic polymer or a synthetic polymer.

19. The composition of claim 16, wherein: the polymer is a biogenic polymer, and wherein the biogenic polymer is a polypeptide-based biogenic polymer, a polynucleotide-based biogenic polymer, or a polysaccharide-based polymer;

optionally wherein (i) the polymer is a polypeptide-based polymer, said polypeptide-based polymer selected from the group consisting of collagen, fibrin, fibrinogen, gelatin, silk, elastin, myosin, keratin, and actin; or

(ii) the polymer is a polysaccharide-based polymer, said polysaccharide-based polymer selected from the group consisting of chitin, chitosan, alginate, hyaluronic acid,

cellulose, agarose, starch, cellulose, dextran, hyaluronic acid, glycogen and glycosaminoglycans, or

wherein the polymer is a synthetic polymer, said synthetic polymer selected from the group consisting of Polycaprolactone, polyglycolic acid, poly lactic acid, polylactic-co-glycolic acid, poly(ethylene oxide) polyethylene glycol, polyurethane,), a poly(siloxane), a poly(ethylene), a poly(vinyl pyrrolidone), a poly(2-hydroxy ethyl methacrylate), a poly(N-vinyl pyrrolidone), a poly(methyl methacrylate), a poly(vinyl alcohol), a poly(acrylic acid), a polyacrylamide, a poly(ethylene-co-vinyl acetate), a poly(ethylene glycol), a poly(methacrylic acid), polyhydroxybutyrate (PHB), Polypropylene fumarate (PPF), a polyvinyl alcohol (PVA), a polypropylene carbonate, a polyanhydride, a polyphosphazene, a polygermane, a polyorthoester, a polyester, a polyamide, a polyolefin, a polycarbonate, a polyaramide, a polyimide,), chitosan, Poly(2 -hydroxyethyl methacrylate) (PHEMA), 2-Hydroxyethyl methacrylate (HEMA), Hydroxyethoxyethyl metha-crylate (HEEMA), Hydroxydiethoxyethylmethacrylate (HDEEMA), Methoxyethyl methacrylate (MEMA), Methoxyethoxyethyl methacrylate (MEEMA), Methoxy-diethoxyethyl methacrylate (MDEEMA), Ethylene glycol dimethacrylate (EGDMA), N-vinyl-2-pyrrolidone (NVP), N-isopropyl Aam (NIPAAm), Vinyl acetate (Vac), Acrylic acid (AA), N-(2-hydroxypropyl) methacrylamide (HPMA), Ethylene glycol (EG), PEG acrylate (PEGA), PEG methacrylate (PEGMA), PEG diacrylate (PEGDA), PEG dimethacrylate (PEGDMA), Methacrylic acid (MAA), PEG-PEGMA, Carboxymethyl cellulose (CMC), Polyvinylpyrrolidone (PVP), an Acrylamide/acrylic acid copolymer, linear cationic poly allyl ammonium chloride, and poly(N- isopropyl acrylamide) (PNIPAM).

20. The composition of claim 16, wherein the polymer forms a hydrogel, optionally wherein the polymer is cross-linked.

21. The composition of claim 16, wherein the TSS further comprises one or more of an extracellular matrix component and an agent.

22. The composition of claim 21, wherein the extracellular matrix component is an extracellular matrix protein, a peptide derived from the extracellular matrix protein or a region or a portion thereof, optionally wherein the extracellular matrix protein is fibronectin, laminin, vitronectin, tenascin, entactin, thrombospondin, elastin, gelatin, collagen, fibrin,

merosin, ankaline, chondronectin, link protein, bone sialo acid protein, osteocalcin, osteopontin, epinectin, hyaluronectin, unjulin, Epiligrin or carinine .

23. The composition of claim 22, wherein the peptide is SEQ ID NO: 9-18.

24. The composition of claim 23, wherein the agent is a biological agent or a chemical agent, optionally wherein the biological agent is a ligand, an immune modulator, or a hormone.

25. The composition of claim 16, further comprising (i) a population of supporting cells, said supporting cells comprising one or more of a myelin cell, a myoid cell, a neuroendocrine cell, a Tuft cell, an ionocyte, an endothelial cell, a mesenchymal stem cell, or a fibroblast, (ii) a population of effector cells and/or (iii) a population of stem cells .

26. The composition of claim 15 or 16, wherein the level of FOXN1 production *in vitro* by a sample of the thymic cell population having a concentration of about 200,000 thymic cells per milliliter is greater than 0.0001 when measured by qRT-PCR and normalized against GADPH.

27. Use of the composition of claim 15 or 16 in the manufacture of a medicament for use in treating or preventing a condition in a subject.

28. The use of claim 27, wherein the condition is associated with the absence, decline or aberrant functioning of the thymus of the subject, such as Di George syndrome, thymoma (such as type A thymoma or type B thymoma), CHARGE syndrome, FOXN1 deficiency, PAX1 deficiency, TBX1 deficiency, thymus cancer, thymic atrophy (such as age-related thymic atrophy), thymic cyst, thymic hyperplasia, thymic hypoplasia, thymic aplasia, thymic dysplasia, thymic irradiation, myasthenia gravis, thymic carcinoma, thymic hyperplasia, thymic irradiation, or age- or infection-associated decline in thymic function.

29. The use of claim 27, wherein the subject has undergone a thymectomy surgery.

30. The use of claim 27, wherein the condition is selected from the group consisting of immunodeficiency, cancer, autoimmune disease, infectious disease, and graft versus host disease (GvHD).

31. Use of the composition of claim 15 or 16 in the manufacture of a medicament for improving acceptance of an organ transplant and/or preventing rejection of an organ transplant in a subject receiving an organ transplant.