SUMMARY

[0006] Provided herein is a method of obtaining human pre-otic epithelial cells. As described herein, the method comprises (a) culturing human pluripotent stem cell aggregates in a culture medium comprising a Bone Morphogenetic Protein (BMP) and an inhibitor of Transforming Growth Factor Beta (TGFβ) signaling for about eight to about 10 days; (b) further culturing the cultured aggregates of (a) in the presence of a Fibroblast Growth Factor (FGF) and an inhibitor of BMP signaling for about 4 days; and (b) contacting the further cultured aggregates of (b) to a Wnt agonist for about 4 days; whereby cells within the contacted aggregates differentiate into pre-otic epithelial cells. In a first aspect, the present disclosure refers to a method of obtaining human pre-otic epithelial cells, comprising, in order, the steps of: (a) culturing human induced pluripotent stem cell aggregates in a culture medium comprising a Bone Morphogenetic Protein 4 (BMP4) and a small molecule inhibitor of Transforming Growth Factor Beta (TGFβ) signaling for four to six days; (b) further culturing the cultured aggregates of (a) in the presence of a Fibroblast Growth Factor (FGF) and a small molecule inhibitor of BMP signaling for 4 days; and (c) contacting the further cultured aggregates of (b) to a Wnt agonist for 4 days, wherein the Wnt agonist is an inhibitor of GSK3; whereby cells within the contacted aggregates differentiate into pre-otic epithelial cells. The FGF can be FGF-2. The BMP can be BMP2, BMP4, or BMP7. The inhibitor of BMP signaling can be LDN-193189. The inhibitor of TGFβ1-mediated signaling can be selected from the group consisting of SB431542 and A-83-01. The Wnt agonist can be an inhibitor of GSK3. The inhibitor of GSK3 is selected from the group consisting of CHIR99021, lithium chloride (LiCl), and 6-bromoindirubin-3'-oxime (BIO).

[0007] In a second aspect, provided herein is a method of obtaining a three-dimensional composition comprising human inner ear sensory tissue, the method comprising the steps of (a) embedding human pre-otic epithelial cells obtained according to the method of the first aspect in a semi-solid culture medium comprising extracellular matrix protein; and (b) culturing the embedded pre-otic epithelial cells in the presence of a Wnt agonist, wherein the Wnt agonist is an inhibitor of GSK3, for 40 to 60 days under conditions that promote self-assembly of embedded pre-otic epithelial cells into otic vesicles, whereby a three-dimensional composition comprising human inner ear sensory tissue is obtained. The Wnt agonist can be an inhibitor of GSK3, where the inhibitor of GSK3 is selected from the group consisting of CHIR99021, lithium chloride (LiCl), and 6-bromoindirubin-3'-oxime (BIO). The extracellular matrix can be a basement membrane extract (BME). The three-dimensional composition can comprise one or more mechanosensory cells. The three-dimensional composition can comprise one or more sensory neuron cells. The three-dimensional composition cam comprise one or more sensory neuron cells that form synaptic connections with mechanosensory cells.

[0008] These and other features, aspects, and advantages described herein will become better understood upon consideration of the following drawings, detailed description, and appended claims.

CLAIMS

We claim:

1. A method of obtaining human pre-otic epithelial cells, comprising, in order, the steps of:

(a) culturing human induced pluripotent stem cell aggregates in a culture medium comprising a Bone Morphogenetic Protein 4 (BMP4) and a small molecule inhibitor of Transforming Growth Factor Beta (TGFβ) signaling for four to six days;

(b) further culturing the cultured aggregates of (a) in the presence of a Fibroblast Growth Factor (FGF) and a small molecule inhibitor of BMP signaling for 4 days; and

(c) contacting the further cultured aggregates of (b) to a Wnt agonist for 4 days, wherein the Wnt agonist is an inhibitor of GSK3; whereby cells within the contacted aggregates differentiate into pre-otic epithelial cells.

2. The method of claim 1, wherein the FGF is FGF-2.

3. The method of claim 1, wherein the inhibitor of BMP signaling is LDN-193189.

4. The method of claim 1, wherein the inhibitor of TGFβ-mediated signaling is selected from the group consisting of SB431542 and A-83-01.

5. The method of claim 1, wherein the inhibitor of GSK3 is selected from the group consisting of CHIR99021, lithium chloride (LiCl), and 6-bromoindirubin-3'-oxime (BIO).

6. A method of obtaining a three-dimensional composition comprising human inner ear sensory tissue, the method comprising the steps of

(a) embedding human pre-otic epithelial cells obtained according to the method of claim 1 in a semi-solid culture medium comprising extracellular matrix protein;

(b) culturing the embedded pre-otic epithelial cells in the presence of a Wnt agonist, wherein the Wnt agonist is an inhibitor of GSK3, for 40 to 60 days under conditions that promote self-assembly of embedded pre-otic epithelial cells into otic vesicles, whereby a three-dimensional composition comprising human inner ear sensory tissue is obtained.

7. The method of claim 6, wherein the inhibitor of GSK3 is selected from the group consisting of CHIR99021, lithium chloride (LiCl), and 6-bromoindirubin-3'-oxime (BIO).

8. The method of claim 6, wherein the extracellular matrix is a basement membrane extract (BME).

9. The method of claim 6, wherein the three-dimensional composition comprises one or more mechanosensory cells.

10. The method of claim 6, wherein the three-dimensional composition comprises one or more sensory neuron cells.

11. The method of claim 6, wherein the three-dimensional composition comprises one or more sensory neuron cells that form synaptic connections with mechanosensory cells.