

In another aspect, the disclosure provides a method for producing a choroid plexus organoid comprising producing a population of neuroepithelial cells from a population of stem cells, and culturing the population of neuroepithelial cells in the presence of a WNT pathway activator and a bone morphogenetic protein (BMP) signalling pathway activator.

- 5 In preferred examples, the population of neuroepithelial cells is an aggregate of neuroepithelial cells.

In preferred examples, the choroid plexus organoid comprises: (a) an epithelium comprising a tight epithelial barrier; and/or (b) one or more cysts surrounded by an epithelium.

- 10 In some examples, the stem cells are embryonic stem cells. In some examples, the stem cells are induced pluripotent stem cells.

- 15 In some examples, culturing the population of neuroepithelial cells in the presence of a WNT pathway activator and a BMP signalling pathway activator is started less than 18 days e.g. between about 8-12 days after starting culturing the population of stem cells. In some examples, culturing the population of neuroepithelial cells in the presence of a WNT pathway activator and a BMP signalling pathway activator is started between about 9-11 days after starting culturing the population of stem cells. In some examples, culturing the population of neuroepithelial cells in the presence of a WNT pathway activator and a BMP signalling pathway activator is started between about 9.5-10.5 days after starting culturing the population of stem cells. In some examples, culturing the population of neuroepithelial cells in the presence of a WNT pathway activator and a BMP signalling pathway activator is started about 10 days after starting culturing the population of stem cells.

In another aspect, the disclosure provides a method for producing a choroid plexus organoid comprising the steps:

- 25 (a) producing a population of embryoid bodies by culturing a population of stem cells;
- (b) culturing the population of embryoid bodies under conditions suitable for neural induction;
- (c) embedding the product of step (b) in a three-dimensional matrix, preferably an extracellular matrix (ECM);
- 30 (d) culturing the product of step (c) in the presence of a Wingless-related (WNT) pathway activator and a bone morphogenetic protein (BMP) signalling pathway activator, preferably wherein step (d) is started between about 8-12 days after the start of step (a).

- 35 In some examples, the stem cells are embryonic stem cells. In some examples, the stem cells are induced pluripotent stem cells.

In some examples, the culturing of step (a) is for about 4-6 days, preferably about 4.5-5.5 days, preferably about 5 days.

Claims

1. A choroid plexus organoid comprising: (a) an epithelium comprising a tight epithelial barrier; and/or (b) one or more cysts surrounded by an epithelium.
2. The choroid plexus organoid of claim 1, wherein the epithelium is transthyretin (TTR)⁺, multidrug resistance protein (MRP1)⁺, aquaporin 1 (Aqp1)⁺ and/or zonula occludens (ZO1)⁺.
3. The choroid plexus organoid of claim 2, wherein the TTR, Aqp1 and/or ZO1 are enriched on the apical side of the epithelium.
4. The choroid plexus organoid of claim 2, wherein the MRP1 is enriched on the apical and basal sides of the epithelium.
5. The choroid plexus organoid of any preceding claim, wherein the epithelium comprises apical microvilli and optionally primary cilia.
6. The choroid plexus organoid of any preceding claim which further comprises (c) stromal cells.
7. The choroid plexus organoid of any preceding claim which is a lateral ventricle choroid plexus organoid.
8. The choroid plexus organoid of any preceding claim, wherein the one or more cysts are filled with liquid, preferably wherein the liquid comprises one or more proteins selected from the group consisting of transthyretin (TTR), clusterin (CLU), apolipoprotein E (APOE), apolipoprotein A4 (APOA4) and lumican (LUM).
- ~~9. Use of a WNT pathway activator and a bone morphogenetic protein (BMP) signalling pathway activator for producing a choroid plexus organoid, wherein the choroid plexus organoid comprises (a) an epithelium comprising a tight epithelial barrier; and/or (b) one or more cysts surrounded by an epithelium.~~
- ~~10. A method for producing a choroid plexus organoid comprising producing a population of neuroepithelial cells from a population of stem cells, and culturing the population of neuroepithelial cells in the presence of a WNT pathway activator and a bone morphogenetic protein (BMP) signalling pathway activator.~~
- ~~11. The method of claim 10, wherein culturing the population of neuroepithelial cells in the presence of a WNT pathway activator and a BMP signalling pathway activator is started between about 8-12 days after starting culturing the population of stem cells.~~
- ~~129.~~ A method for producing a choroid plexus organoid comprising the steps:
 - (a) producing a population of embryoid bodies by culturing a population of stem cells;
 - (b) culturing the population of embryoid bodies under conditions suitable for neural induction;

- (c) embedding the product of step (b) in a three-dimensional matrix, preferably an extracellular matrix (ECM);
- (d) culturing the product of step (c) in the presence of a Wingless-related (WNT) pathway activator and a bone morphogenetic protein (BMP) signalling pathway activator, ~~preferably wherein step (d) is started between about 8-12 days after the start of step (a).~~

~~43~~10. The method of claim ~~42~~9, wherein the culturing of step (d) comprises agitation.

~~44~~11. The ~~use or the~~ method of any one of claims 9-~~43~~10, wherein the WNT pathway activator is selected from the group consisting of CHIR99021, Wnt1, Wnt2, Wnt3, Wnt3a, Wnt8a, Wnt8b, Wnt10a, Wnt10b, BML-284, 6-bromoindirubin-3'-oxime (BIO), WAY-316606, IQ1, QS11, SB-216763, LY2090314, DCA, 2-amino-4-[3,4-(methylenedioxy)benzyl-amino]-6-(3-methoxyphenyl)pyrimidine and lithium, preferably wherein the WNT pathway activator is CHIR99021.

~~45~~12. The ~~use or the~~ method of any one of claims 9-~~44~~11, wherein the BMP signalling pathway activator is selected from the group consisting of Bmp4, Bmp2, Bmp3, Bmp5, Bmp6, Bmp7, Bmp8a, Bmp8b, Bmp10, Bmp11, Bmp15, isoliquiritigenin, 4'-hydroxychalcone, apigenin, diosmetin, and a ventromorphins (e.g. SJ000291942, SJ000063181 and/or SJ00037178), preferably wherein the BMP signalling pathway activator is Bmp4.

~~46~~13. Use of the choroid plexus organoid of any one of claims 1-8 for drug discovery screening or assaying drug toxicity.

~~47~~14. The use of claim ~~46~~13, wherein the use is for testing the ability of a candidate agent to cross the blood-cerebrospinal fluid (CSF) barrier.

~~48~~15. A method for testing the ability of a candidate agent to cross the blood-cerebrospinal fluid (CSF) barrier, comprising contacting the choroid plexus organoid of any one of claims 1-8 with the candidate agent and determining if the candidate agent crosses the epithelium.