

exhibits the same selectivity to small molecules as the *in vivo* counterpart, and that ChP-CSF organoids can predict CNS permeability of novel compounds.

5 Thus, the organoids provided by the inventors' protocol recapitulate fundamental functions of the choroid plexus, namely secretion and formation of a tight epithelial barrier. The inventors determined that the organoids provided by their protocol reliably and reproducibly develop choroid plexus epithelium with polarised cells that actively secrete a fluid within the organoid with properties closely resembling cerebrospinal fluid (CSF).

10 Furthermore, the inventors discovered the presence of choroid plexus-specific water channels and transporters localised on the apical brush border of the choroid plexus organoid epithelium using histological and electron microscopy (EM) analysis. These tissues were found to display tight junctions forming the epithelial barrier, and the inventors observed the formation of large fluid-filled cysts protruding from the organoids, the contents of which, analysed by mass spectrometry, highly resemble human embryonic CSF.

15 The disclosure provides a tool to study, for example, choroid plexus function, CSF production, and the permeability of compounds, peptides and serum-derived proteins into the human brain through the CSF.

20 Accordingly, in one aspect the disclosure provides a choroid plexus organoid comprising: (a) an epithelium comprising a tight epithelial barrier; and (b) one or more cysts filled with a liquid and surrounded by the epithelium.

In some examples, the choroid plexus organoid comprises an epithelium comprising a tight epithelial barrier. In some examples, the choroid plexus organoid comprises one or more cysts surrounded by an epithelium. In preferred examples, the choroid plexus organoid comprises an epithelium comprising a tight epithelial barrier and one or more cysts
25 surrounded by the epithelium.

In preferred examples, the epithelium is cuboidal epithelium.

In some examples the choroid plexus organoid further comprises (c) stromal cell populations.

In some examples the choroid plexus organoid has a lateral ventricle identity.

30 In preferred examples, the choroid plexus organoid is LUM+, DCN+, and DLK1+.

In some examples, the choroid plexus organoid is TTR+, MRP1+, Aqp1+ and/or ZO1+. In preferred examples, the choroid plexus organoid is TTR+, MRP1+, Aqp1+ and ZO1+.

In other examples, the choroid plexus organoid is TTR+, MRP1+, MRP4+, Aqp1+ and/or ZO1+. In other examples, the choroid plexus organoid is TTR+, MRP1+, MRP4+, Aqp1+ and
35 ZO1+.

In some examples, the choroid plexus organoid comprises at least 50% TTR+ tissue. In some examples, the choroid plexus organoid comprises at least 60% TTR+ tissue. In some examples, the choroid plexus organoid comprises at least 70% TTR+ tissue. In some

- In preferred examples, the cysts comprise a liquid. In preferred examples, 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). In some examples, the liquid is a CSF.
- 5 In some examples, the liquid comprises alphafetoprotein (AFP).
- In some examples, the liquid comprises developmental-specific CSF proteins e.g. Insulin-like growth factor 2 (IGF2), IGFBP7, and/or Follistatin-like protein 1 (FSTL1).
- In some examples, the choroid plexus organoid comprises less than 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2% or 1% (v/v) forebrain neuronal tissue. In some examples, the choroid
- 10 plexus organoid comprises substantially no forebrain neuronal tissue.
- In some examples, the choroid plexus organoid comprises less than 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2% or 1% (v/v) FOXG1+ tissue. In some examples, the choroid plexus organoid comprises substantially no FOXG1+ tissue.
- In some examples, the choroid plexus organoid comprises extracellular matrix (ECM).
- 15 In some examples, the choroid plexus organoid have primary cilia, for example, with microvilli apically located, tight junctions between cells, multi-vesicular bodies and extracellular vesicles.
- In some examples, the choroid plexus organoid is a human or mouse organoid, preferably a human organoid.
- 20 In some examples, the choroid plexus organoid is obtainable by the method of the disclosure.
- In some examples, the choroid plexus organoid is produced from stem cells, optionally embryonic stem cells or induced pluripotent stem cells.
- In another example, the disclosure provides 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.
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- Although there are previous reports of using Bmp4 and/or CHIR (see (15) and (16)) these earlier publications did not yield ChP organoids having the authentic characteristics demonstrated herein.
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- In another example, the disclosure provides a method for producing a choroid plexus organoid comprising contacting a population of neuroepithelial cells with a WNT pathway activator and a bone morphogenetic protein (BMP) signalling pathway activator, wherein the
- 35 choroid plexus organoid comprises: (a) an epithelium comprising a tight epithelial barrier; and/or (b) one or more cysts surrounded by an epithelium.
- The method may be performed with or without fibrous microscavolds. The absence of microscavolds may be preferred.

In another example, 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.

- 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.
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In another aspect, the disclosure provides a method for producing the choroid plexus organoid as described herein comprising the steps:

- 25 (a) producing a population of embryoid bodies by culturing a population of stem cells comprising human embryonic stem cells or induced pluripotent stem cells;
- (b) culturing the population of embryoid bodies under conditions suitable for neural induction;
- 30 (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).
- 35 (e) adding dissolved ECM to the culture medium, preferably wherein the ECM is dissolved Matrigel, preferably wherein step (e) is started between about 30 days after the start of step (a)

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

- 40 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.

Figure 2. Organoids reproduce histological features of human choroid plexus. (A) Representative confocal images of H1 organoids at day 30 and 46 stained for Krt18 (green, anti-mouse Alexafluor 488), 5HT-2C (magenta, anti-rabbit Alexafluor 568), PH3 (grey, anti-rat Alexafluor 647) and DAPI in blue. Scale bar: 50µm. (B) Confocal images of ChP epithelium and stromal tissue from organoids staining positive for TTR (magenta) and DLK1 (green, anti-rabbit Alexafluor 488). Scale bar: 100µm. (C) UMAP showing the population architecture identified by scRNAseq of all combined samples (telencephalic organoid collected at day 55, ChP organoids collected at day 27, 46 and 53). (D) UMAPs showing progressive enrichment in ChP populations in the treated organoids compared to telencephalic organoid. (E) UMAPs showing enrichment of genes involved in ChP development and maturation (CLIC6, HRT2C, MSX1, PAX6), the cortical neuron marker DCX and the stromal marker COL1A1 in the identified scRNAseq clusters. (F) Violin plots showing expression levels of ChP immature/hem (OTX2, RSPO3, PAX6), mature (TTR, KRT18, CA2, NME5, KCNJ13, CA12) and stromal markers (LUM, DCN, DLK1) identified by scRNAseq analysis.

Figure 3. Choroid plexus organoids develop specialized epithelial features and closely recapitulate genetic architecture of human *in vivo* tissue. (A) PCA of E18.5 mouse embryonic whole ChP and isolated ChP epithelial cells (ECs) with organoid clusters from scRNA-seq. (B) Pearson's correlation analysis of *in vivo* human samples with organoid clusters. (C) Color-coded heatmap showing unbiased clustering of organoid with *in vivo* human samples. (D) Pearson's correlation analysis of mouse, human and organoid samples. (E) Violin plots showing expression levels of regional ChP markers for lateral (LV, LY6E), third (3V, INS) and fourth (4V, PENK) ventricle. (F) Violin plots showing expression of DYNLRB2, FOXJ1 and AQP1 in organoid cell populations identified by scRNAseq. (G) Confocal images of H1 ChP organoids at day 40 and d46 respectively stained for CCDC67 (green, anti-rabbit Alexafluor 488), TTR (grey, anti-sheep Alexafluor 647), Foxj1 (magenta, anti-mouse Alexafluor 568), Arl13b (green, anti-rabbit Alexafluor 488) and DAPI in blue. Scale bar: 50µm and 100µm (zoom-in: 20µm). (H) Confocal images of H1 ChP organoid at day 40 and *in vivo* ChP from a mouse embryo (E18.5) staining positive for Aqp1 (magenta, anti-rabbit Alexafluor 568) and TTR (green, anti-sheep Alexafluor 488), Nuclei in blue are stained for DAPI. Scale bar: 50µm. (I) Electron microscopy images showing extensive microvilli on the apical side of ChP epithelium from organoids. Arrowheads indicate cilia (C), tight junctions (TJ), multivesicular bodies (MVB) and extracellular vesicles (Ex). Scale bar: 1µm. (J) Violin plots showing expression levels of APOE, CLU, PLTP in organoid cell clusters identified by scRNAseq. (K) Representative confocal images of H1 organoids at d30 and d46 stained for lipid droplets (LipidTox, grey), TTR (magenta, anti-sheep Alexafluor 568), PH3 (green, anti-rat Alexafluor 488) and DAPI in blue. Scale bar: 50µm.

Figure 4. Choroid plexus organoids develop fluid-filled compartments and form a tight barrier. (A) Violin plots showing expression levels of claudin 1, 3 and 5 in organoid cell clusters identified by scRNAseq. (B) UMAPs showing different organoid populations and

enrichment in tight junction proteins in ChP immature and mature clusters. Visualization of tight junction proteins TJP1 and TJP2, occludin (OCLN), INADL and MPDZ. (C) Representative images of human H1 ChP organoids at day 40 staining positive for TTR (magenta, anti-sheep Alexafluor 568), claudin 1 (green, anti-rabbit Alexafluor 488) claudin 3 (green, anti-rabbit Alexafluor 488) claudin 4 (red, anti-mouse Alexafluor 568) and claudin 5 (green, anti-rabbit Alexafluor 488). Nuclei in blue are labeled with DAPI. Scale bar: 50µm. (D) Representative bright field images of untreated forebrain organoids and of ChP organoids that developed fluid-filled compartments. Scale bar: 1 cm. Zoomed bright field image of a ChP organoid that developed self-contained fluid compartment. Scale bar: 1 mm. (E) Confocal images of the epithelium surrounding the organoid fluid-filled compartment staining positive for ZO1 (grey, anti-mouse Alexafluor 647), TTR (green, anti-sheep 488), Aqp1 (red, anti-rabbit Alexafluor 568). Nuclei are labeled in blue with DAPI. Scale bar: 50µm.

Figure 5. Human developmental proteins are secreted by choroid plexus organoids. (A) gProfileR analysis of proteins detected in iCSF from at least 2 organoid batches showing significant ($p < 0.05$) enrichments for GO categories cellular component (GO:CC), molecular function (GO:MF) and biological process (GO:BP), REAC, WP, HP databases. (B) Color-coded heatmap showing relative abundance (emPAI values) of proteins detected in organoid iCSF from at least two organoid batches (3 H9 batches, one H1 and one iPSCs batch) and their corresponding values in media and in *in vivo* CSF samples pool which includes human adult CSF (hCSF, telencephalic CSF, $n=3$ healthy donors, Caltag-Medsystem), bovine fetal CSF (bCSF, BioIVT) and embryonic mouse CSF (msCSF, E12.5-13.5). Values shown are means of proteins with $\text{emPAI} \geq 1$. (C) Violin plots of secreted proteins IGFBP7 and SERPINF1 expression levels in organoid cell clusters identified by scRNAseq analysis. (D) UMAPs showing enrichments in secreted proteins RBP1, IGF2, NPC2 and the specific transporters SLC23A2 and SLC46A1 in different cell populations identified by organoid scRNAseq. (E) Two color-coded heatmaps (emPAI values): on the top, abundant proteins with an $\text{emPAI} \geq 1$ detected only in human-derived samples (organoid iCSF and adult hCSF); on the bottom, abundant developmentally related proteins with an $\text{emPAI} \geq 1$ detected only in organoid iCSF and in embryonic or fetal *in vivo* CSF. (F) Immunoblot validation of IGF2 and TTR in organoid total lysate from H9 untreated and treated, ChP organoids, media and iCSF from 3 independent batches. (G) Representative confocal images of ChP epithelium from organoids staining positive for IGF2 (green, anti-rabbit Alexafluor 488) and TTR (grey, anti-sheep Alexafluor 647). Nuclei are labeled with DAPI in blue. Scale bar: 100µm. (H) Venn diagram plot of CSF proteins present in human early embryonic (Carnegie stage 20), adult and ChP organoid.

Figure 6. Choroid plexus organoids model human brain barrier permeability. (A) Relative quantifications of NMR spectra of Dopa and L-Dopa in media and iCSF. (B) Relative NMR spectra quantifications of bupropionyl, methotrexate and vincristine in media and iCSF after 2h incubation. (C) Relative quantifications of NMR spectra of sephin 1 in media and iCSF after 2h incubation. (D) Representative images of ChP epithelium from organoids staining positive for TTR (grey, anti-sheep Alexafluor 647), LAT1 (magenta, anti-mouse Alexafluor 568), Pgp (green, anti-rabbit Alexafluor 488), transporters MRP4 (green, anti-rabbit Alexafluor 488) and MRP1 (green, anti-rabbit Alexafluor 488). Scale bar: 50µm. Nuclei are labeled in blue with DAPI. (E) Pharmacokinetic model showing correlation ($R^2=0.9921$; Slope=1.004) between *in vivo* CSF/plasma ratio ($K_{p,uu,CSF}$) and *in vitro*

iCSF/media ratio ($K_{m,uu,iCSF}$) of unbound drugs (Supplementary Table 5). Sephin 1 (red dot) *in vivo* CSF/plasma measurements are reported in mice to have a much greater value than that detected in human organoids (45). (F) Relative quantifications of NMR spectra of BIA 10-2474 in media and iCSF after 2h incubation. (G) Time course analysis of the ratio of BIA 10-2474 and bupropionyl in iCSF to media at 2h, 12h, 24h and 72h. (All drugs were tested in n=3 independent experiments; **Supplementary Table 2**). (H) Schematic diagram to show that ChP organoids may be exploited as a human *in vitro* pre-clinical model to predict quantitatively the permeability of unknown compounds and also their dynamics over time.

Figure 7. (A) Confocal images of a forebrain organoid with a convoluted, cuboidal ChP epithelium staining positive for the markers TTR, ZO1 and Aqp1. Scale bar: 100 μ m. (B) Comparison of treated, ChP organoids versus untreated, forebrain organoids at day 14. Arrowheads point to the elongated neuroepithelial buds in early treated ChP organoids. Scale bar: 1 mm. (C) Bright field images of different stages of the protocol using the fiber seeding method. Scale bar 1000 μ m. (D) Representative confocal images of H9 ChP organoids generated using the round and the fiber method, at day 30 and 34 respectively. Scale bar: 500 μ m. (E) Representative confocal images of iPSC IMR90-4 organoids at day 35 and of H1 organoids at day 40, treated with BMP4 and CHIR at day 10. ChP tissue in the organoids is stained for TTR (green, anti-sheep Alexafluor 488) and nuclei are visualized with DAPI. Scale bar: 1000 μ m (crop: 500 μ m).

Figure 8. (A) Representative confocal images of H1 forebrain and ChP organoids stained for Sox2 (magenta, anti-rabbit Alexafluor 568), the cortical intermediate progenitor marker Tbr2 (grey, anti-sheep Alexafluor 647), the neuronal marker HuC/D (green, anti-mouse Alexafluor 488). Scale bar: 100 μ m. (B) Representative confocal images of H1 ChP organoids stained for DCN (green, anti-mouse Alexafluor 488), LUM (magenta, anti-rabbit Alexafluor 568), ZO1 (grey, anti-mouse Alexafluor 647), TTR (grey, anti-sheep Alexafluor 647) and DAPI in blue. Scale bar: 100 μ m.

Figure 9. (A) Representative bright field images of 4 independent batches of H1 untreated and BMP4+CHIR treated organoids (from top to bottom: 57, 54, 46, 40 days). (B) Time course series of images of H1 treated ChP organoids that show development and expansion of iCSF compartments from day 14 to day 50. Scale bar: 1000 μ m. (C) Immunoblot for ChP epithelial transporters MRP1, LAT1, CLIC6, secreted protein IGF2, and the loading control GAPDH of ChP organoid lysates from day 10 to day 31.

Figure 10 (A) Venn diagram of proteins detected by mass spectrometry with an emPAI ≥ 1 in at least one sample in media, organoid iCSF and *in vivo* CSF samples. (B) Color-coded heatmap of the same proteins in **Fig. 3E** showing all samples analysed by mass spectrometry. Proteins with emPAI ≥ 1 detected in at least two iCSF samples are shown. (C) Color-coded heatmap of abundant proteins (emPAI ≥ 1) excluded or absent from the organoid iCSF, but present in at least two *in vivo* samples. (D) Color-coded heatmap showing abundant proteins (emPAI ≥ 1) shared between media and iCSF (mean of five samples shown) from at least two organoid batches and the mean of the *in vivo* CSF samples from human adult CSF (hCSF), bovine fetal CSF (bCSF) and embryonic mouse CSF (msCSF, E12.5-13.5). (E) gProfileR analysis of proteins detected only in *in vivo* CSF samples. (F) Histogram showing top categories from gProfileR analysis with significant ($p < 0.05$) enrichment.

Figure 11. (A - top) ChP epithelium from human organoids stained for TTR (grey, anti-sheep Alexafluor 647), claudin 2 (green, anti-rabbit Alexafluor 488) and APOE (magenta, anti-mouse Alexafluor 568). Scale bar: 50µm. DAPI in blue labels the nuclei. (A - bottom) Staining for tight junction marker occludin (green, anti-rabbit Alexafluor 488), TTR (grey, anti-sheep Alexafluor 647), ZO1 (magenta, anti-mouse Alexafluor 568) and DAPI in blue of ChP epithelium from human organoids. Scale bar: 50µm. (B) Bright field image of ChP organoid with self-contained iCSF fluid, isolated from media incubated for 2h with 647-Alexafluor labeled 10kDa-dextran. (C) Fluorescent intensity measured in media and iCSF after 2h incubation with 70kDa Oregon green-dextran, 10kDa Alexa 647-dextran and 3-5kDa FITC-dextran. (D) NMR spectra of Dopa, L-Dopa and control (Ctrl) iCSF measured after 2h incubation.

Figure 12. (A) ¹H-NMR analysis (H₂O/D₂O) of the control samples (media and iCSF). Expansions of the spectra are reported for clarity. (B) ¹H-NMR analysis (H₂O/D₂O) of samples upon incubation with L-Dopa (500µM) and Carbidopa (500µM). (C) ¹H-NMR analysis (H₂O/D₂O) of samples upon incubation with Dopamine (500µM) (500µM). Arrows indicate the peaks of interest.

Figure 13. (A) ¹H-NMR analysis (H₂O/D₂O) of the samples (media and iCSF) upon incubation with (A) Sephin 1 (500µM), (B) Vincristine (500µM), (C) Methotrexate (500µM). Expansions of the spectra are reported for clarity. Arrows indicate the peaks of interest.

Figure 14. (A) ¹H-NMR analysis (H₂O/D₂O) of the samples (media and iCSF) upon incubation with Bupropionyl (500µM). Expansions of the spectra are reported for clarity. Arrows indicate the peaks of interest.

Figure 15. (A) ¹H-NMR analysis (H₂O/D₂O) of the samples (media and iCSF) upon incubation with BIA10-2474 (500µM). Expansions of the spectra are reported for clarity. Arrows indicate the peaks of interest.

DETAILED DESCRIPTION OF THE INVENTION

The terms “comprising”, “comprises” and “comprised of” as used herein are synonymous with “including” or “includes”; or “containing” or “contains”, and are inclusive or open-ended and do not exclude additional, non-recited members, elements or steps. The terms “comprising”, “comprises” and “comprised of” also include the term “consisting of”.

Organoid

Organoids are three-dimensional (3D) in vitro cell cultures. Organoids may provide a miniaturised and simplified version of an organ, which incorporate some key features of the organ. These in vitro culture systems may contain a self-renewing stem cell population that differentiates into multiple, organ-specific cell types with spatial organisation similar to the organ.

Choroid plexus

The choroid plexus is a highly conserved secretory tissue in the brain, which displays a number of important functions, such as forming a protective epithelial barrier and secreting the cerebrospinal fluid (CSF).

In vivo, ChP epithelium initially develops as a pseudostratified epithelium, followed by an intermediate columnar stage, and maturing into a highly folded cuboidal epithelium (1, 18, 19). To confirm tissue specificity, we performed staining for the ChP marker TTR, the neural stem cell marker Sox2 and the tight junction marker ZO1 at early and late time points (**Fig. 1E, Fig. 1F**). ChP organoids treated with Bmp4 and CHIR developed a pseudostratified neuroepithelium with TTR positive areas by day 28 (**Fig. 1E**). By day 39, treated organoids developed a more columnar, polarised ChP epithelium, increasingly enriched in TTR immunostaining, with relatively strong ZO1 expression in cell-cell junctions, and sparse Sox2 immunostaining (**Fig. 1F**). Quantification of TTR-positive regions showed a reproducible enrichment in treated organoids compared to untreated controls (**Fig. 1G**). Together, these data demonstrate a reliable and reproducible generation of ChP tissue *in vitro* from different lines of human pluripotent stem cells and with two different seeding methods.

The ChP expresses the highest levels in the brain of the channel protein-chloride intracellular channel (CLIC6) (20). We could detect CLIC6 in ChP organoid epithelium both by immunohistochemistry and by immunoblot compared with control, untreated organoids. In parallel, lower levels of forebrain marker Foxg1 were detected in treated organoids compared to controls. Cortex-specific Tbr2 positive intermediate progenitors and HuC/D positive neurons were also largely reduced in early ChP organoids and almost completely absent in mature ChP organoids compared to controls (**Fig. 8A**).

The ChP contains both epithelial and stromal components: the polarized epithelium apically faces the lumen of the ventricle and surrounds a stromal core, exposed to the fenestrated capillaries. To better characterize the different cell populations present in the ChP organoids, we performed single cell RNA-sequencing (scRNA-seq) sampling two organoids from three separate batches of ChP organoids at different maturation stages and two untreated telencephalic organoids as a control (**Fig. 2C, Fig. 2D**). Combined analysis identified the expected clusters of cells expressing markers of neurons and progenitors of neurons in untreated organoids, while immature and mature ChP identities were enriched in ChP organoids (**Fig. 2C**). Unbiased analysis revealed a consistent and progressive enrichment over time in ChP populations in the treated organoids (**Fig. 2D**). Older treated organoids displayed a larger component of ChP mature epithelial cells as well as enrichment in the stromal cell population compared to earlier stage organoids and the untreated control (**Fig. 2D**). Consistent with our previous stainings, we confirmed the expression of CLIC6 and of other specific ChP markers such as the serotonin receptor HRT2C (21) (**Fig. 2E**). As expected, the immature ChP/hem cluster of cells expressed higher levels of early regulatory factors involved in ChP development such as homeobox protein MSX1, OTX2, R-spondin 3 (RSPO3) and PAX6 (**Fig. 2E, Fig. 2F**). Mature ChP cell populations expressed higher levels of TTR, carbonic anhydrase 2 and 12 (CA2, CA12), nucleoside diphosphate kinase homolog 5 (NME5), inward rectifier potassium channel 13 (KCNJ13) and of the epithelial marker keratin 18 (Krt18) (**Fig. 2F**). A gradient of maturation in the ChP epithelium *in vivo*, with more mature Krt18 positive cells positioned distally into the lateral ventricle, has been previously reported (21). Krt18 was enriched in more mature ChP organoids compared to early, immature ones (**Fig. 2A**). To investigate the stromal component of the ChP in our system, we looked at the expression of DLK1 (delta-like noncanonical notch ligand 1), a gene expressed in ChP mesenchyme (21), by immunohistochemistry (**Fig. 2B**). Expression of DLK1 and of other ChP stromal proteins (lumican, LUM and decorin, DCN) was detected in

areas of the organoid surrounding ChP and within the epithelium (**Fig. 2B, 8B**). In agreement with these results, the ChP stromal population detected by scRNA-seq expressed high levels of DCN, LUM and DLK1 (**Fig. 2F**), as well as the extracellular matrix component collagen alpha-1 chain 1 (COL1A1) (**Fig. 2E**). These data suggest that *in vitro* derived ChP organoids develop both epithelial and stromal components.

We next sought to identify similarities between our *in vitro* model and the ChP tissue *in vivo*. We performed unbiased clustering and principal component analysis (PCA) with published datasets of mouse ChP tissue (1) and developing human brain (2) (**Fig. 3A-D**). First, we performed PCA between our identified organoid clusters and RNAseq data from *in vivo* E18.5 mouse embryonic whole ChP and isolated epithelial ChP cells (4). We could observe the closest proximity between ChP from *in vivo* samples and our mature ChP cluster compared with other identified clusters (**Fig. 3A**). We then performed correlation analysis between the organoid clusters and those of *in vivo* human developing brain (22) revealing a high correlation between organoid and *in vivo* ChP clusters, and between organoid and *in vivo* stromal clusters (**Fig. 3B**). Further unbiased hierarchical clustering revealed clustering of organoid mature and immature ChP together with *in vivo* ChP, and organoid ChP stroma clustering with *in vivo* stromal identity (**Fig. 3C**). To further investigate whether our system could better model specifically human ChP, we then compared the organoid mature ChP cluster to both *in vivo* human and mouse (**Fig. 3D**) revealing a higher correlation with human ChP than with mouse (**Fig. 3D**). Finally, examination of specific regional ChP markers (4, 24) revealed the choroid plexus organoids to be of lateral ventricle identity rather than third or fourth ventricle (**Fig. 3E**) consistent with their telencephalic origin. These data overall demonstrate that the ChP epithelial and stromal composition of the organoids seems to recapitulate the cell composition and genetic architecture of the human ChP *in vivo*.

To examine the extent of maturation of ChP organoids we looked at previously described markers of ChP maturation. We could observe the presence of ciliary markers, suggested to be expressed during ChP epithelial maturation (3), as well the marker of more mature ChP epithelium aquaporin 1 (AQP1). We could detect an enrichment in cilia genes such as DYNLRB2 and FOXJ1 in the mature ChP cluster, as well as enrichment in AQP1 (**Fig. 3F**). Staining further revealed the presence of CCDC67 and FOXJ1, both regulators of ciliogenesis, and the ciliary protein ARL13B which stained tufts of apical cilia in the ChP epithelium (**Fig. 3G**). Staining for AQP1 also confirmed the presence of this more mature marker, showing localization on the apical side of the epithelium, similar to *in vivo* (**Fig. 3H**). The ChP epithelium is an extremely efficient secretory machinery (23) with numerous microvilli enhancing its surface area on the apical side, motile cilia and a dense cytoplasmic vesicular network. To further investigate whether ChP organoids recapitulate these fundamental ultrastructural features we performed EM imaging. We observed primary cilia, extensive microvilli apically located, tight junctions between cells, multi-vesicular bodies and numerous extracellular vesicles (**Fig. 3I**; indicated by arrowheads). In line with its roles in brain lipid metabolism, we also observed an enrichment in lipid droplets, and lipoproteins (ApoE, clusterin/ApoJ, and phospholipid transfer protein PLTP) (**Fig. 3J**). These features along with a loss of actively cycling cells positive for the cell cycle marker phosphohistone H3 (PH3) (**Fig. 3K**), suggest a quite mature secretory epithelium in ChP organoids.

Next, we asked whether ChP organoids could develop a tight barrier by examining the expression of tight junctions markers such as claudins and occludin which seal the

intercellular spaces in between cells of the ChP epithelial barrier (25, 26). The scRNA-seq revealed the presence of several of the claudins (**Fig. 4A**) as well as other tight junction components occludin (OCLN), ZO1 (TJP1), ZO2 (TJP2), and PDZ proteins PATJ (INADL) and MPDZ (**Fig. 4B**). Staining revealed apical localization of claudin 1, 3, 4 and 5 in the ChP organoid polarized epithelium and validated their expression enrichment (**Fig. 4C**). Low levels of claudin 2 were also observed in ChP organoid epithelium as well as the presence of OCLN (**Fig. 11A**). Finally, to directly assess the barrier function of the ChP epithelium we applied fluorescently labeled dextrans of varying molecular weights (70, 10 and 3-5kDa) and examined their entry into the organoids. After 2h incubation, no apparent leakage of 10kDa Alexa-647 labeled dextrans was visible inside the organoid (**Fig. 11B**). Fluorescent intensity of media and internal fluid collected after 2h incubation with 70kDa, 10kDa and 3-5kDa labeled dextrans confirmed the complete exclusion of these molecules from the organoid (**Fig. 11C**).

A striking feature of ChP organoids was the later development of large fluid-filled compartments or cysts containing a colorless liquid, which were rarely present or completely absent in forebrain, untreated organoids (**Fig. 4D, Fig. 9A-B**). We found that these compartments were completely isolated from the media and could be maintained with continued addition of dissolved Matrigel to the media. We observed the same cuboidal epithelium surrounded these compartments and displaying polarized orientation with apical markers Aqp1 and ZO1 on the luminal side towards the fluid (**Fig. 4D**).

To investigate the composition of this fluid we performed mass spectrometry analysis to compare *in vitro* organoid CSF-like fluid (iCSF) (3 batches of H9, 1 batch of H1 and 1 batch of iPSCs IMR-90), with 3 different *in vivo* samples (human adult telencephalic CSF pooled from 3 healthy donors, mouse embryonic CSF at E13.5, and fetal bovine CSF) as well as media. We detected in total 248 abundant proteins in organoid iCSF of which 199 were in common with *in vivo* CSF samples (**Fig. 10A**). Gene Ontology Analysis revealed that the GO:CC categories “extracellular vesicle” and “extracellular space”, and the GO:BP categories “export from cell” and “secretion from cell” were enriched in the organoid fluid proteome (**Fig. 5A**). Insulin-like growth factors, particularly IGF2, are secreted by the ChP and regulate cortical progenitor proliferation in developmental CSF (2). Consistent with the role of IGFs in developmental CSF, REAC category “Regulation of Insulin-like Growth Factor” was also significantly enriched in organoid iCSF.

We next focused on proteins detected in at least 2 organoid iCSF samples in order to rule out technical artifacts, and we then examined the most abundant proteins with a reported emPAI value of 1, based on the number of observed peptides. Of these 83 abundant proteins detected in organoid iCSF, 80 were also detected in *in vivo* samples, further pointing to a high similarity (**Fig. 5B, 10C, 10D**). Among these proteins, we detected several biomarkers such as clusterin (CLU), phospholipid transfer protein (PLTP) and apolipoprotein E (APOE) (**Fig. 5B, Supplementary Table 1**). Many of the proteins detected in iCSF, such as Insulin-like growth factor binding protein 7 (IGFBP7), Serpin Family F Member 1 (SERPINF1), Niemann-Pick disease type C2 protein (NPC2) and retinol-binding protein 1, could also be detected in the scRNA-seq ChP clusters (**Fig. 5C, Fig. 5D**) further validating their production. A subset of proteins were only present in the human organoid and adult human CSF samples suggesting potentially human-specific secretion (**Fig. 5E**). We also detected several developmental-specific CSF proteins, including Insulin-like growth factor 2

(IGF2), IGFBP7, and Follistatin-like protein 1 (FSTL1), absent in the adult human sample, indicating that the iCSF secreted by the ChP organoid retains at least some developmental features of human CSF (**Fig. 5E**). IGF2 levels in organoid fluid were validated by immunoblot (**Fig. 5G, Fig. 8C**), and immunohistochemistry revealed its presence in the ChP epithelium (**Fig. 5H**). Consistent with the unique role of ChP in the transport of folate and vitamin C, both vital for brain homeostasis and function (27, 28), we detect enrichment of specific transporters involved in the trafficking of these nutrients: SLC23A2 (vitamin C) and SLC46A2 (folate) in ChP epithelial clusters (**Fig. 5D**).

We next asked what stage the iCSF from organoids best matches by further comparing with a previously published proteomics dataset of human embryonic Carnegie Stage 20 CSF (29). We could observe a larger overlap of organoid iCSF with the adult CSF, with 121 proteins in common compared to 51 proteins shared between iCSF and embryonic *in vivo* CSF (**Fig. 5E**), indicating that iCSF is probably more similar to adult CSF but still retains some developmental features.

Overall, there was very good agreement between the *in vitro* and the *in vivo* samples; however, in order to fully ascertain the similarity to *in vivo*, we further examined the proteins with unusual distributions. First, while the vast majority of proteins detected were not present in the media, pointing to a highly selective barrier, proteomic analysis revealed that of the 14 abundant proteins in the media, 13 were also present in organoid iCSF (**Fig. 10C, 10D**). Importantly, most of these were also detected in *in vivo* samples, and included proteins such as albumin (ALB) and transferrin (TF), which are actively transported across the ChP epithelium. Those not detected *in vivo* are proteins provided by the Matrigel including several laminins. Second, there were several proteins detected reproducibly in the *in vivo* samples but not in the organoid iCSF that warranted further examination as these could be factors that the organoid failed to properly produce. However, we found that proteins detected exclusively in the *in vivo* CSF samples but not in the organoid iCSF showed an enrichment in GO:CC “blood microparticle”, GO:BP “platelet degranulation” and KEGG: “Complement and coagulation cascade” (**Fig. 10B, 10E, 10F**). This could be explained by the fact that the invasive procedure of lumbar puncture (spinal tap) CSF collection often results in contamination with peripheral blood and bone marrow. Thus, one of the advantages of the organoid system is that ChP organoids lack the vascular component; therefore, we expect this fluid to be exclusively ChP secretome. Together, these findings suggest that: 1. ChP organoids develop self-contained compartments, distinct from the surrounding media by a tight, epithelial barrier, 2. ChP organoids actively secrete CSF-like fluid enriched in human, developmental-specific CSF proteins, free of blood contaminants.

As part of its selective barrier function, the ChP epithelium is also highly selective to small molecules, including therapeutics. Thus, in order to test for such selective permeability to small molecules, we applied various therapeutically relevant small molecules and assayed their permeability by NMR analysis of organoid iCSF. As a proof of principle, we tested the permeability of the barrier to dopamine (Dopa) and its precursor, Levodopa (L-Dopa), since these are highly similar molecules with very different permeability *in vivo*, and such specificity has not previously been reported in any *in vitro* models of the BBB. It is well established that Dopa doesn't enter the brain *in vivo*, whereas L-Dopa is actively transported in to the CSF via the LAT-1 transporter (30–33) and is therefore given as the therapeutic agent in dopamine replacement therapy in patients with Parkinson's disease. We incubated

the organoids with Dopa and with L-Dopa in the presence of Carbidopa, a small molecule that prevents the conversion of L-Dopa into Dopa (34, 35). After 2h, we could detect the presence of both L-Dopa and Dopa in the media but only L-Dopa was detectable in the iCSF, consistent with the *in vivo* properties of a CNS barrier (**Fig. 6A, Fig. 11E**). L-Dopa iCSF/media ratio was consistently higher compared to Dopa iCSF/media ratio suggesting that our model exhibited the same selectivity to small molecules as *in vivo* (**Fig. 6A**).

These findings suggest there may be proper expression of key transporters in the blood-CNS barrier. Indeed, we were able to detect expression of the transporter LAT-1 on the apical side of the ChP organoid epithelium (**Fig. 6D, Fig. 9C**), which explains the proper transport of L-Dopa seen in our permeability assay. We also stained for the well-known efflux pump P-glycoprotein 1 (Pgp or multidrug resistance protein 1, MDR1), which stops drugs from entering the brain via ATP-dependent mechanisms (36, 37) (**Fig. 6D**). We detected apical and subapical expression of Pgp, which seemed to localize to intracellular vesicles, in ChP epithelium (**Fig. 6D**). Pgp localization in ChP has not been well-established (38, 39), however, it is reported to have a role in pumping molecules from the CSF to the blood (38, 40). Additional important efflux transporters in the ChP are MRP1 and MRP4, which prevent toxic compounds and unwanted signals from reaching the CSF (38, 41). MRP1 was both apically and basally localized on the ChP epithelium at 40 days in culture (**Fig. 6D, Fig. 9C**), while MRP4 was apically expressed in ChP epithelium (**Fig. 6D**). These results suggest that *in vitro* generated ChP epithelium endogenously expresses transport and export pumps that are critical for the correct function of the B-CSF-B in human brain.

We next tested whether the system could quantitatively predict drug permeability. For this purpose, we performed similar permeability experiments to test bupropionyl, an antidepressant known to readily cross the BBB (42), and two chemotherapeutic compounds, methotrexate and vincristine, which poorly cross the BBB (43, 44). As predicted, bupropionyl was readily detectable in the iCSF after 2h, while methotrexate and vincristine were completely excluded from the iCSF (**Fig. 6B**). Most importantly, we found a very high correlation between *in vitro* iCSF/media ratios and the *in vivo* CSF/plasma ratios reported in the literature for the same drugs ($R^2=0.9921$). Not only that but the actual values corresponded remarkably well (as indicated by a slope of 1.004), indicating that this *in vitro* system can qualitatively and quantitatively recapitulate the permeability of drugs reported *in vivo* (**Fig. 6E**).

These results suggest that the ChP organoid system could be used to model and predict CNS permeability of unknown drugs. To test this theory, we turned to Sephin 1, a novel inhibitor of the regulatory subunit PPP1R15A of protein phosphatase 1, which has been shown to cross the BBB and to have a protective CNS role against protein misfolding in a mouse model of Charcot-Marie Tooth-1B (45). Pre-clinical or clinical data of this molecule in humans are not yet available, but Phase I clinical trials are under way so any additional human *in vitro* data would be informative. Using the same experimental conditions as previously described, sephin 1 was detectable iCSF after 2h incubation, indicating that this compound could also cross the human CNS barrier (**Fig. 6C**). Notably, however, the permeability in the human organoid model was much lower than that described in the mouse model, suggesting a higher dose may be needed in humans to achieve the same CNS levels.

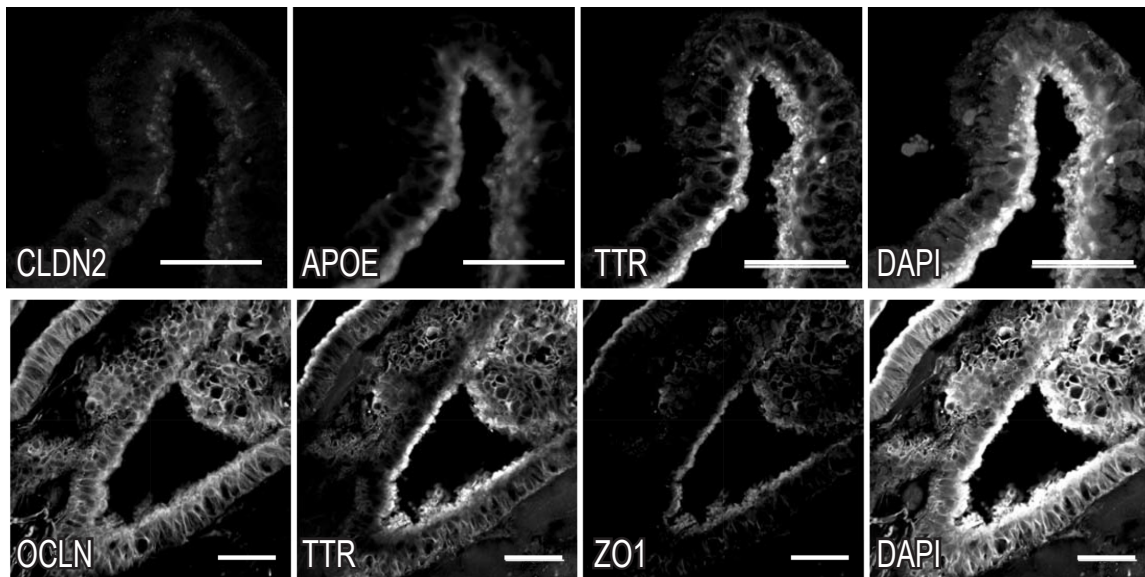
Claims

1. A choroid plexus organoid comprising: (a) an epithelium comprising a tight epithelial barrier; and (b) one or more cysts filled with a liquid and surrounded by the 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 an apical side of the epithelium and a basal side 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 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. A method for producing the choroid plexus organoid of any one of claims 1 to 8, comprising the steps:
 - (a) producing a population of embryoid bodies by culturing a population of stem cells comprising human embryonic stem cells or induced pluripotent 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;
 - (e) adding dissolved ECM to the culture medium, preferably wherein the ECM is dissolved Matrigel, preferably wherein step (e) is started between about 30 days after the start of step (a).

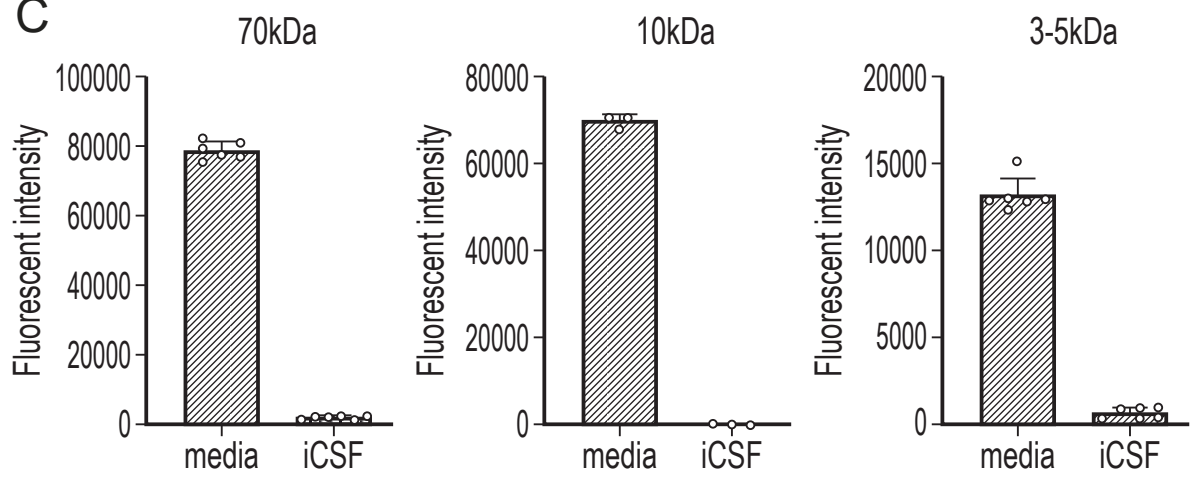
10. The method of claim 9, wherein the culturing of step (d) comprises agitation.
11. The method of any one of claims 9-10, wherein the WNT pathway activator is selected from the group consisting of CHIR99021, Wnt1, Wnt2, Wnt3, Wnt3a, Wnt8a, Wnt8b, Wnt10a, Wnt10b, BML-284, 6-bromoindirubin-3'-oxime (BIO), WAY-316606, QS11, SB-216763, LY2090314, 2-amino-4-[3,4-(methylenedioxy)benzyl-amino]-6-(3-methoxyphenyl)pyrimidine and lithium, preferably wherein the WNT pathway activator is CHIR99021.
12. The method of any one of claims 9-11, 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 ventromorphin; preferably wherein the BMP signalling pathway activator is Bmp4; preferably wherein the ventromorphin is SJ000291942, SJ000063181 and/or SJ00037178.
13. Use of the choroid plexus organoid of any one of claims 1-8, for drug discovery screening or assaying drug toxicity.
14. The use of claim 13, wherein the use is for testing the ability of a candidate agent to cross the blood-cerebrospinal fluid (CSF) barrier.
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.

24/28

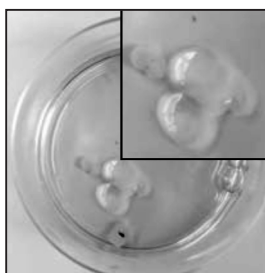
A



C



B



D

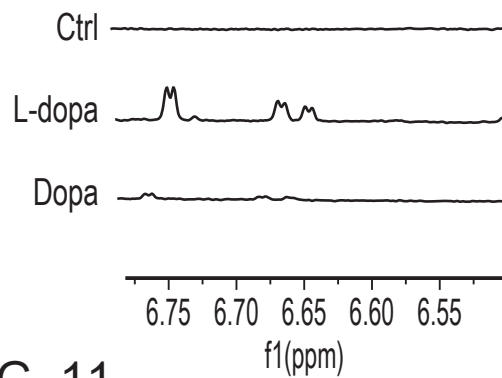


FIG. 11