CLAIMS

1. A method of obtaining an intestinal organoid-derived monolayer comprising:

    i. digesting or dissociating one or more intestinal organoids into a suspension of single cells and/or organoid fragments;

    ii. seeding a semi-permeable membrane with said suspension;

    iii. culturing the cells and/or organoid fragments in the presence of an expansion medium until a monolayer is formed; and

    iv. culturing the monolayer in the presence of a differentiation medium comprising a Notch inhibitor, an EGFR pathway inhibitor and a Wnt agonist.

2. The method of claim 1, wherein:

    (i) the monolayer is cultured in the presence of an expansion medium until it reaches transepithelial electrical resistance (TEER) of about 100 $\Omega \cdot cm^2$;

    (ii) TEER of the monolayer further increases during the step of culturing the monolayer in the presence of the differentiation medium, optionally wherein TEER of the monolayer reaches more than 500, more than 600, more than 700, more than 800, more than 900, more than 1000, more than 1100, more than 1200, more than 1300, more than 1400 or more than 1500 $\Omega \cdot cm^2$ during the step of culturing the monolayer in the presence of the differentiation medium;

    (iii) the expansion medium comprises a receptor tyrosine kinase ligand, a BMP inhibitor and a Wnt agonist and, optionally, nicotinamide and a p38 MAPK inhibitor, such as SB202190, further optionally wherein:

        (a) the receptor tyrosine kinase ligand is a ligand for RTK class I (EGF receptor family) (ErbB family), a ligand for RTK class II (Insulin receptor family), a ligand for RTK class IV (FGF receptor family) or a ligand for RTK class VI (HGF receptor family), further optionally wherein the receptor tyrosine kinase ligand is selected from the group consisting of: epidermal growth factor (EGF), neuregulin, fibroblast growth factor (FGF), hepatocyte growth factor (HGF) and insulin-like growth factor (IGF); and/or

        (b) the BMP inhibitor is selected from the group consisting of noggin, sclerostin, chordin, CTGF, follistatin, gremlin, tsg, sog, LDN193189 or dorsomorphin;

(iv) the Wnt agonist is selected from the group consisting of: Rspondin, Wnt conditioned medium and Wnt surrogate;

(v) the Notch inhibitor is a gamma secretase inhibitor, optionally selected from the group consisting of: DAPT, dibenzazepine (DBZ), benzodiazepine (BZ) and LY-411575;

(vi) the EGFR pathway inhibitor is selected from: (1) an EGFR inhibitor, such as Gefitinib, (2) an EGFR and ErbB2 inhibitor, such as Afatinib, (3) an inhibitor of the RAS-RAF-MAPK pathway, (4) an inhibitor of the PI3K/AKT pathway and (5) an inhibitor of the JAK/STAT pathway, optionally wherein the EGFR pathway inhibitor is an inhibitor of the RAS-RAF-MAPK pathway, e.g. a MEK inhibitor, such as PD0325901; and/or

(vii) the monolayer is cultured in the presence of:

(a) an expansion medium for at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days or at least 10 days, preferably wherein the monolayer is cultured in the presence of an expansion medium for 3-9 days;

(b) the differentiation medium for at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 or more, preferably wherein the monolayer is cultured in the presence of a differentiation medium for 4-8 days; and/or

(c) an extracellular matrix.

3. An intestinal organoid-derived monolayer obtainable or obtained by the method of claim 1 or claim 2.

4. The method or organoid-derived monolayer of any one of claims 1-3, wherein:

(i) the monolayer comprises one or more of the following cell types: Lgr5+ stem cell, enterocyte, goblet cell, Paneth cell and enteroendocrine cell; and/or

(ii) the monolayer is derived from a mammal, optionally wherein the monolayer is derived from a human, further optionally wherein the human has a disease or disorder of the digestive system, such as inflammatory bowel disease (e.g. Crohn's disease or ulcerative colitis), coeliac disease or leaky gut syndrome.

5. A method of obtaining a lung organoid-derived monolayer comprising:

i. digesting or dissociating one or more lung organoids into a suspension of single cells and/or organoid fragments;

ii. seeding a semi-permeable membrane with said suspension; and

iii. culturing the cells and/or organoid fragments in the presence of an expansion medium until a monolayer is formed.

6. The method of claim 5, wherein:

(i) the method further comprises:

iv. culturing the monolayer in the presence of a differentiation medium;

(ii) the monolayer is cultured in the presence of an extracellular matrix;

(iii) the expansion medium comprises one or more receptor tyrosine kinase ligands, a Wnt agonist, a TGF-beta inhibitor a BMP inhibitor and, optionally, a Rho kinase inhibitor, such as Y-27632, and/or a p38 MAPK inhibitor, such as SB202190, further optionally wherein:

(a) the receptor tyrosine kinase ligand is a ligand for RTK class I (EGF receptor family) (ErbB family), a ligand for RTK class II (Insulin receptor family), a ligand for RTK class IV (FGF receptor family) or a ligand for RTK class VI (HGF receptor family), further optionally wherein the receptor tyrosine kinase ligand is selected from the group consisting of: epidermal growth factor (EGF), neuregulin, fibroblast growth factor (FGF), hepatocyte growth factor (HGF) and insulin-like growth factor (IGF);

(b) the BMP inhibitor is selected from the group consisting of noggin, sclerostin, chordin, CTGF, follistatin, gremlin, tsg, sog, LDN193189 or dorsomorphin;

(c) the Wnt agonist is selected from the group consisting of: Rspondin, Wnt conditioned medium and Wnt surrogate; and/or

(d) the TGF-beta inhibitor is selected from the group consisting of: A83-01, SB-431542, SB-505124, SB-525334, LY 364947, SD-208 and SJN 2511;

(iv) the differentiation medium comprises one or more receptor tyrosine kinase ligands, a Wnt agonist, a Notch inhibitor, a BMP pathway activator and, optionally, a Rho kinase inhibitor, such as Y-27632, and/or a p38 MAPK inhibitor, such as SB202190, further optionally wherein:

(a) the receptor tyrosine kinase ligand is a ligand for RTK class I (EGF receptor family) (ErbB family), a ligand for RTK class II (Insulin receptor family), a ligand for RTK class IV (FGF receptor family) or a ligand for RTK class VI (HGF

receptor family), further optionally wherein the receptor tyrosine kinase ligand is selected from the group consisting of: epidermal growth factor (EGF), neuregulin, fibroblast growth factor (FGF), hepatocyte growth factor (HGF) and insulin-like growth factor (IGF);

(b) the Wnt agonist is selected from the group consisting of: Rspondin, Wnt conditioned medium and Wnt surrogate;

(c) the Notch inhibitor is a gamma secretase inhibitor, optionally selected from the group consisting of: DAPT, dibenzazepine (DBZ), benzodiazepine (BZ) and LY-411575; and/or

(d) the BMP pathway activator is selected from the group consisting of BMP7, BMP4 and BMP2;

(v) the method comprises seeding the semi-permeable membrane with less than about 20,000 cells, less than about 30,000 cells, less than about 40,000 cells, less than about 50,000 cells, less than about 60,000 cells, less than about 70,000 cells, less than about 80,000 cells, less than about 90,000 cells, less than about 100,000 cells, or less than about 250,000 cells, for example, in a standard 96-well format;

(vi) the method comprises seeding the semi-permeable membrane with about 30,000 cells, about 40,000 cells, about 50,000 cells, about 60,000 cells, about 70,000 cells, about 80,000 cells or about 90,000 cells, for example, in a standard 96-well format;

(vii) the method comprises seeding the semi-permeable membrane with about 5,000-500,000 cells, about 10,000-250,000 cells, about 20,000-100,000 cells, about 30,000-50,000 cells, about 35,000-45,000 cells, or preferably about 40,000 cells, for example, in a standard 96-well format;

(viii) the method comprises adjusting the suspension of single cells and/or organoid fragments to less than about 0.2 x 10^6 cells per mL, less than about 0.3 x 10^6 cells per mL, less than about 0.4 x 10^6 cells per mL, less than about 0.5 x 10^6 cells per mL, less than about 10^6 cells per mL, less than about 2 x 10^6 cells per mL, less than about 3 x 10^6 cells per mL, less than about 4 x 10^6 cells per mL or less than about 5 x 10^6 cells per mL before seeding;

(ix) the method comprises adjusting the suspension of single cells and/or organoid fragments to about 0.2 x 10^6 cells per mL, about 0.3 x 10^6 cells per mL, about 0.4 x 10^6

cells per mL, about 0.5 x 10^6 cells per mL, about 10^6 cells per mL, about 2 x 10^6 cells per mL, about 3 x 10^6 cells per mL, about 4 x 10^6 cells per mL or about 5 x 10^6 cells per mL before seeding;

(x) the method comprises adjusting the suspension of single cells and/or organoid fragments to about 0.1-1 x 10^6 cells per mL, about 0.25-0.75 x 10^6 cells per mL, about 0.3-0.5 x 10^6 cells per mL, about 0.35-0.45 x 10^6 cells per mL, preferably about 0.4 x 10^6 cells per mL before seeding;

(xi) the monolayer is cultured in the presence of:

(a) an expansion medium for at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, at least 15 days, at least 16 days or more, preferably wherein the monolayer is cultured in the presence of an expansion medium for 3-8 days; and/or

(b) a differentiation medium for at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 or more, preferably wherein the monolayer is cultured in the presence of a differentiation medium for 8 days;

and/or

(xii) the method further comprises removing the expansion or differentiation medium from the apical compartment, optionally wherein the medium is removed from the apical compartment 10-16 days after seeding, for example, 11 days, 12 days, 13 days, 14 days or 15 days, preferably 13 days, after seeding.

7. A lung organoid-derived monolayer obtained or obtainable by the method of claim 5 or claim 6.

8. The method or organoid-derived monolayer of any one of claims 5-7, wherein:

(i) the monolayer comprises one or more of the following cell types: club cells, basal cells, ciliated cells, goblet cells, alveolar type I cells and alveolar type II cells; and/or

(ii) the monolayer is derived from a mammal, for example a human.

9. A method of obtaining a kidney organoid-derived monolayer comprising:

i. digesting or dissociating one or more kidney organoids into a suspension of single cells and/or organoid fragments;

ii. seeding a semi-permeable membrane with said suspension; and

iii. culturing the cells and/or organoid fragments in the presence of an expansion medium until a monolayer is formed.

10. The method of claim 9, wherein:

(i) the method further comprises:

iv. culturing the monolayer in the presence of a differentiation medium;

(ii) the monolayer is cultured in the presence of an extracellular matrix;

(iii) the expansion medium comprises one or more receptor tyrosine kinase ligands, a Wnt agonist, and a TGF-beta inhibitor and, optionally, a Rho kinase inhibitor;

(iv) the method comprises seeding the semi-permeable membrane with less than about 100,000 cells, less than about 150,000 cells, less than about 200,000 cells, or less than about 250,000 cells, for example, in a standard 96-well format;

(v) the method comprises seeding the semi-permeable membrane with about 30,000 cells, about 40,000 cells, about 50,000 cells, about 60,000 cells, about 70,000 cells, about 80,000 cells, about 90,000 cells, or about 100,000 cells, for example, in a standard 96-well format;

(vi) the method comprises seeding the semi-permeable membrane with about 20,000-500,000 cells, about 30,000-400,000 cells, about 40,000-300,000 cells, about 50,000-250,000 cells, about 60,000-200,000 cells, about 70,000-150,000 cells, about 80,000-120,000 cells, or preferably about 100,000 cells, for example, in a standard 96-well format;

(vii) the method comprises adjusting the suspension of single cells and/or organoid fragments to less than about 0.5 x 10^6 cells per mL, less than about 0.6 x 10^6 cells per mL, less than about 0.7 x 10^6 cells per mL, less than about 0.8 x 10^6 cells per mL, less than about 0.9 x 10^6 cells per mL, less than about 10^6 cells per mL, less than about 1.1 x 10^6 cells per mL, less than about 1.2 x 10^6 cells per mL, less than about 1.3 x 10^6 cells per mL, less than about 1.4 x 10^6 cells per mL or less than about 1.5 x 10^6 cells per mL before seeding;

(viii) the method comprises adjusting the suspension of single cells and/or organoid fragments to about 0.2 x 10^6 cells per mL, about 0.3 x 10^6 cells per mL, about 0.4 x 10^6 cells per mL, about 0.5 x 10^6 cells per mL, about 10^6 cells per mL, about 1.5 x 10^6 cells per

mL, about 2 x 10^6 cells per mL, about 3 x 10^6 cells per mL, about 4 x 10^6 cells per mL or about 5 x 10^6 cells per mL, preferably about 10^6 cells per mL, before seeding;

(ix) the method comprises adjusting the suspension of single cells and/or organoid fragments to about 0.1-5 x 10^6 cells per mL, about 0.25-2.5 x 10^6 cells per mL, about 0.5-1.5 x 10^6 cells per mL, about 0.75-1.25 x 10^6 cells per mL, about 0.8-1.2 x 10^6 cells per mL, preferably about 10^6 cells per mL, before seeding;

(x) the monolayer is cultured in the presence of:

(a) an expansion medium for at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days or more, preferably wherein the monolayer is cultured in the presence of an expansion medium for 1-3 days, more preferably 2 days; and/or

(b) a differentiation medium for at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days or more, preferably wherein the monolayer is cultured in the presence of a differentiation medium for 3-5 days, more preferably 4 days;

and/or

(xi) the method further comprises adding a histone deacetylase inhibitor, such as decitabine, to the expansion or differentiation medium, optionally wherein the histone deacetylase inhibitor is added 1-3 days after seeding, preferably 2 days after seeding.

11. A kidney organoid-derived monolayer obtained or obtainable by the method of claim 9 or claim 10.

12. The kidney organoid-derived monolayer of claim 11, wherein the monolayer has TEER of more than 25, more than 50, more than 75, more than 100, more than 200, more than 300, more than 400, more than 500, more than 600, more than 700, more than 800, more than 900, more than 1000, more than 1100, more than 1200, more than 1300 or more than 1400 $\Omega \cdot cm^2$.

13. The method or organoid-derived monolayer of any one of claims 9-12, wherein:

(i) the monolayer comprises one or more of the following cell types: proximal tubule cells, kidney epithelial cells, loop of Henle cells, distal tubule cells and collecting duct cells; and/or

(ii) the monolayer is derived from a mammal, for example a human.

14. Use of an organoid-derived monolayer according to any one of claims 3, 4, 7, 8, and 11-13 in an assay assessing epithelial viability, metabolic activity, permeability, barrier function integrity and/or activity of transporter proteins.

15. A method of identifying a compound capable of modulating epithelial viability, metabolic activity, permeability, barrier function integrity and/or activity of transporter proteins comprising:

    i. contacting an organoid-derived monolayer, for example according to any one of claims 3, 4, 7, 8, and 11-13, with one or more candidate molecules; and

    ii. assessing the viability, metabolic activity, permeability and/or barrier function integrity of the organoid-derived monolayer and/or activity of transporter proteins in the organoid-derived monolayer.

16. A method of assessing the effect of a compound on epithelial viability, metabolic activity, permeability, barrier function integrity and/or activity of transporter proteins comprising:

    i. contacting an organoid-derived monolayer, for example according to any one of claims 3, 4, 7, 8, and 11-13, with said compound; and

    ii. assessing the viability, metabolic activity, permeability and/or barrier function integrity of the organoid-derived monolayer and/or activity of transporter proteins in the organoid-derived monolayer.

17. The method of claim 15 or claim 16, wherein the method further comprises contacting the organoid-derived monolayer with one or more proinflammatory cytokines, optionally wherein the one or more proinflammatory cytokines are selected from the group consisting of: IFN-γ, TNF-α and IL-1α.

18. A method of identifying a mutation associated with epithelial viability, metabolic activity, permeability, barrier function integrity and/or activity of transporter proteins comprising:

    i. assessing the viability, metabolic activity, permeability and/or barrier function integrity of an organoid-derived monolayer and/or activity of transporter proteins in an organoid-derived monolayer, for example an organoid monolayer according to any one of claims 3, 4, 7, 8, and 11-13; and

    ii. determining the presence of one or more mutations in the genome of one or more cells in the organoid-derived monolayer.

19. A method of diagnosing a disease or affliction that affects epithelial viability, metabolic activity, permeability, barrier function integrity and/or activity of transporter proteins, or determining an increased risk of said disease or affliction, in a human subject comprising:

    i. obtaining an organoid-derived monolayer from said human subject as described in any one of claims 1, 2, 4-6, 8-10 and 13; and

    ii. testing the viability, metabolic activity, permeability and/or barrier function integrity of the organoid-derived monolayer and/or activity of transporter proteins in the organoid-derived monolayer,

    wherein a test result above or below a reference value indicates the presence of, or an increased risk of, said disease or affliction in the human subject.

20. The method of claim 19, wherein:

    (i) the reference value is a value obtained from a control, e.g. an organoid-derived monolayer obtained from a healthy human subject; and/or

    (ii) the disease or affliction is a disease or disorder of the digestive system, such as inflammatory bowel disease (e.g. Crohn's disease or ulcerative colitis), coeliac disease or leaky gut syndrome.

21. A method of predicting the likelihood of a patient's response to a candidate compound comprising:

    i. obtaining an organoid-derived monolayer from said patient as described in any one of claims 1, 2, 4-6, 8-10 and 13;

    ii. contacting the organoid-derived monolayer with said compound; and

    iii. assessing the viability, metabolic activity, permeability and/or barrier function integrity of the organoid-derived monolayer and/or activity of transporter proteins in the organoid-derived monolayer.

22. The use or method of any one of claims 14-21, wherein:

    (i) assessing the barrier function integrity of the organoid-derived monolayer comprises measuring TEER of the organoid-derived monolayer;

    (ii) assessing the permeability of the organoid-derived monolayer comprises measuring the rate of passive diffusion of a reporter compound across the monolayer, optionally wherein said reporter compound is a dye, optionally a fluorescent dye, such as Lucifer yellow;

(iii) assessing the activity of transporter proteins comprises measuring the rate of transport of a substrate of a transporter protein across the monolayer, optionally in the presence of an inhibitor of said transporter protein, optionally wherein the substrate is a dye, such as Rhodamine 123 or Calcein AM; and/or

(iv) assessing the activity of transporter proteins comprises measuring the rate of transport of a substrate of a transporter protein into the cells of the monolayer, optionally in the presence of an inhibitor of said transporter protein, optionally wherein the substrate is a dye, such as Rhodamine 123 or Calcein AM.