Claims

1. A crypt-villus organoid, comprising a central lumen lined by a villus-like epithelium or a colon crypt organoid.

2. A method for culturing epithelial stem cells, isolated tissue fragments comprising said epithelial stem cells, or adenoma cells, the method comprising

providing an extracellular matrix;

incubating an epithelial stem cell, an isolated tissue fragment comprising said epithelial stem cells, or an adenoma cell with the extracellular matrix;

culturing the stem cell, isolated tissue fragment, or adenoma cell in the presence of a cell culture medium, comprising a basal medium for animal or human cells to which is added:

a Bone Morphogenetic Protein (BMP) inhibitor;

between 5 and 500 ngram/ml of a mitogenic growth factor,

whereby a Wnt agonist is added if epithelial stem cells and isolated tissue fragments are cultured.

3. A method according to claim 2, in which the BMP inhibitor is Noggin, the mitogenic growth factor is Epidermal Growth Factor and Keratinocyte Growth Factor, and the Wnt agonist is R-spondin 1.

4. A method of claim 2, wherein said BMP inhibitor is selected from Noggin, DAN, and DAN-like proteins including Cerberus and Gremlin. .

5. A method according to claim 2 or 4, wherein said Wnt agonist is selected from one or more of Wnt, R-spondin 1-4, Norrin, and a GSK-inhibitor.

6. A method according to claims 2 and 3-5, wherein said Wnt agonist comprises R-spondin 1 and Wnt-3a and/or said mitogenic growth factor is EGF.

7. A method of any one of claims 2 to 6, wherein said culture medium further comprises a Rock (Rho-kinase) inhibitor, selected from Y-27632, Fasudil, and H-1152.

8. A method according to any of claims 2 to 7, wherein said culture medium further comprises a notch agonist.

9. A method for obtaining and/or culturing a colon crypt , preferably according to any one of claims 2 to 8 ,wherein:
- epithelial stem cells, isolated tissue fragments comprising said epithelial stem cells or adenoma cells are cultured in contact with an extracellular matrix in a medium comprising Noggin, EGF, and R-spondin 1 and/or Wnt-3 as Wnt agonist, supplemented with B27, N2, and N-Acetylcystein.

10. A method preferably according to any one of claims 2 to 8, for obtaining and/or culturing a pancreatic organoid wherein:
- epithelial stem cells, isolated tissue fragments comprising said epithelial stem cells or adenoma cells are cultured in contact with an extracellular matrix in a first step in a medium comprising EGF, KGF or FGF, and R-spondin 1 as Wnt agonist, supplemented with B27, N2, and N-Acetylcystein ,
- subsequently in a second step in a medium comprising EGF and R-spondin 1 as Wnt agonist, supplemented with B27, N2, and N-Acetylcystein.

11. A method for obtaining and/or culturing a gastric fragment , preferably according to any one of claims 2 to 8, wherein:
- epithelial stem cells, isolated tissue fragments comprising said epithelial stem cells or adenoma cells are cultured in contact with an extracellularmatrix in a first step in a medium comprising Noggin as BMP inhibitor, Epidermal Growth Factor and FGF10 as mitogenic growth factor, R-spondin 1 and Wnt-3a as Wnt agonist, and further comprising B27, N2, N-Acetylcystein,
- subsequently in a second step in a medium comprising Epidermal Growth Factor and R-spondin 1 and Wnt-3a as Wnt agonist, Noggin and FGF10 supplemented with B27, N2, and N-Acetylcystein, wherein the concentration of Wnt-3 is reduced in the second step by comparison to the Wnt-3a concentration as present in the first step.

12. A cell culture medium, comprising a basal medium for animal or human cells to which is added:

a Bone Morphogenetic Protein (BMP) inhibitor;

a Wnt agonist; and

between 5 and 500 nanogram/ml of Epidermal Growth Factor (EGF).

13. Use of a culture medium according to claim 12 for culturing epithelial stem cells, or isolated tissue fragments on an extracellular matrix.

14. A crypt-villus organoid, comprising a central lumen lined by a villus-like epithelium or a colon crypt organoid obtainable by a method as defined in claims 2 to 9.

15. A pancreatic organoid, comprising islet-like structures, preferably obtainable by a method as defined in claims 2 to 8 or 10.

16. A gastric organoid, comprising a central lumen, preferably obtainable by a method as defined in claims 2 to 8 or 11.

17. Use of the crypt-villus organoids or colon crypt of claim 1 or 14, the pancreatic organoids of claim 15, or the gastric organoids of claim 16 in a drug discovery screen, toxicity assay, or in regenerative medicine.

18. Use of a cell culture medium comprising a basal medium for animal or human cells to which is added:

a Bone Morphogenetic Protein (BMP) inhibitor; and

between 5 and 500 nanogram/ml of Epidermal Growth Factor (EGF)

for culturing adenoma cells.

We claim:

1. A method for culturing epithelial stem cells or isolated tissue fragments comprising said epithelial stem cells, the method comprising

providing an extracellular matrix;

incubating an epithelial stem cell or an isolated tissue fragment comprising said epithelial stem cells with the extracellular matrix;

culturing the epithelial stem cell or isolated tissue fragment in the presence of a cell culture medium comprising a basal medium for animal or human cells to which is added:

a Bone Morphogenetic Protein (BMP) inhibitor; and

between 5 and 500 nanogram/ml of a mitogenic growth factor.

2. The method of claim 1, wherein the cell culture medium additionally comprises a Wnt agonist.

3. A method according to claim 1 or claim 2, wherein the epithelial stem cells are normal epithelial cells or adenoma epithelial cells, and whereby a Wnt agonist is added if normal epithelial cells are cultured.

4. A method according to any one of claims 1 to 3, wherein said BMP inhibitor is selected from Noggin, DAN, and DAN-like proteins.

5. A method according to claim 4, wherein said BMP inhibitor is Cerberus or Gremlin.

6. A method according to any one of claims 1 to 5, wherein said Wnt agonist is selected from one or more of Wnt, any one of R-spondin 1-4, Norrin, and a GSK-inhibitor.

7. A method according to any one of claims 1 to 6, wherein said Wnt agonist comprises any one of R-spondin 1-4 and/or Wnt-3a.

8. A method according to any one of claims 1 to 7, wherein said mitogenic growth factor is selected from the group consisting of epidermal growth factor (EGF), transforming growth factor-alpha (TGF-alpha), fibroblast growth factor (FGF), brain derived neurotrophic factor (BDNF) and keratinocyte growth factor (KGF).

9. A method according to claim 8, wherein said mitogenic growth factor is EGF.

10. A method according to any one of claims 1 to 9, in which the BMP inhibitor is Noggin, the mitogenic growth factor is Epidermal Growth Factor, and the Wnt agonist is any one of R-spondin 1-4.

11. A method of any one of claims 1 to 10, wherein said culture medium further comprises a Rock (Rho-kinase) inhibitor.

12. The method of claim 11, wherein the Rock inhibitor is selected from Y-27632, Fasudil, and H-1152.

13. A method of any one of claims 1 to 12, wherein said culture medium further comprises a Notch agonist.

14. A cell culture medium, comprising a basal medium for animal or human cells to which is added:

a Bone Morphogenetic Protein (BMP) inhibitor; a mitogenic growth factor; and

a Wnt agonist.

15. The cell culture medium of claim 14, wherein between 5 and 500 nanogram/ml of mitogenic growth factor are added to the basal medium.

16. A cell culture medium, according to claim 15, wherein the mitogenic growth factor is selected from one or more mitogenic growth factors in the group consisting of EGF, TGF-alpha, FGF, BDNF, and KGF.

17. The cell culture medium of claim 16, wherein the mitogenic growth factor is EGF.

18. The cell culture medium according to any one of claims 14 to 17, wherein the Wnt agonist is selected from any one of R-spondin 1-4, and/or wherein the BMP inhibitor is Noggin.

19. A cell culture medium according to claim 18, wherein the medium is supplemented with at least one of B27, N2 and N-Acetylcysteine

20. A cell culture medium according to claim 14 or 19, wherein the BMP inhibitor is Noggin, the mitogenic growth factor is EGF and the Wnt agonist is any one of R-spondin 1-4.

21. A cell culture medium according to any one of claims 14 to 20 in combination with an extracellular matrix.

22. The cell culture medium according to claim 21, wherein the extracellular matrix comprises a collagen and laminin.

23. The cell culture medium according to any one of claims 21 to 22, wherein the culture medium is added to or embedded in the extracellular matrix.

24. Use of a culture medium according to any one of claims 14 to 19, for culturing epithelial stem cells, isolated tissue fragments or isolated organoid structures on or in an extracellular matrix.

25. Use according to claim 24, wherein the epithelial stem cells are normal epithelial cells or adenoma epithelial cells.

26. A method for culturing a three dimensional tissue organoid in the presence of an extracellular matrix, wherein the method comprises culturing the organoid in a culture medium according to any one of claims 14 to 23.

27. The method of claim 26, wherein said three dimensional tissue organoid is cultured in a medium comprising Noggin and EGF.

28. The method according to claim 27, wherein the medium additionally comprises R-spondin 1 and/or Wnt-3a as Wnt agonist.

29. A method for obtaining an organoid by culturing colon or small intestinal epithelial stem cells, or tissue fragments comprising said epithelial stem cells, using a method according to any one of claims 1 to 13.

30. The method of claim 29, wherein the step of culturing comprises culturing said colon or small intestinal epithelial stem cells, or tissue fragments comprising said epithelial stem cells, in contact with an extracellular matrix in a medium comprising Noggin, EGF, and R-spondin 1 and/or Wnt-3a as Wnt agonist, supplemented with B27, N2, and N-Acetylcystein.

31. The method of claim 29 or 30, wherein the tissue fragments comprising said epithelial stem cells are isolated colon crypts or small intestinal crypts.

32. A method for obtaining a pancreatic organoid by culturing pancreatic epithelial stem cells, or tissue fragments comprising said epithelial stem cells, using a method according to any one of claims 1 to 13.

33. The method of claim 32, wherein the step of culturing comprises

- culturing said pancreatic epithelial stem cells or tissue fragments comprising said epithelial stem cells in contact with an extracellular matrix in a first step in a medium comprising EGF, KGF or FGF, and R-spondin 1 as Wnt agonist, supplemented with B27, N2, and N-Acetylcystein, and

- subsequently in a second step in a medium comprising EGF and R-spondin 1 as Wnt agonist, supplemented with B27, N2, and N-Acetylcystein.

34. A method for obtaining a gastric organoid by culturing gastric epithelial stem cells, or tissue fragments comprising said epithelial stem cells, using a method according to any one of claims 1 to 13.

35. The method of claim 34, wherein the step of culturing comprises

- culturing said gastric epithelial stem cells or tissue fragments comprising said epithelial stem cells in contact with an extracellular matrix in a first step in a medium comprising

Noggin as BMP inhibitor, Epidermal Growth Factor and FGF10 as mitogenic growth factor, R-spondin 1 and Wnt-3a as Wnt agonist, and further comprising B27, N2, NAcetylcystein, and

- subsequently in a second step in a medium comprising Epidermal Growth Factor and R-spondin 1 and Wnt-3a as Wnt agonist, Noggin and FGF10 supplemented with B27, N2, and N-Acetylcystein, wherein the concentration of Wnt-3a is reduced in the second step by comparison to the Wnt-3a concentration as present in the first step.

36. A three-dimensional organoid obtainable by a method as defined in any one of claims 1 to 13 or any one of claims 27 to 35.

37. A three dimensional organoid comprising a central lumen, wherein the organoid is derived from intestinal epithelial stem cells, pancreatic epithelial stem cells, or gastric epithelial stem cells.

38. A three-dimensional organoid of claim 36, wherein the organoid is derived from intestinal epithelial stem cells, pancreatic epithelial stem cells, or gastric epithelial stem cells.

39. A three dimensional organoid, according to claim 36 or 37, comprising crypt-like domains surrounding a central lumen lined by villus-like epithelial domains, which are epithelial domains comprising differentiated cell types, and wherein all the cells of the organoid are epithelial cells.

40. A three dimensional organoid according to claim 36 or 37 comprising pancreatic islet-like structures, which are epithelial domains comprising differentiated cell types, and wherein all the cells of the organoid are epithelial cells.

41. The three-dimensional organoid according to claim 36 or 37, wherein the organoid is derived from one or more adenoma cells and has a balloon-like structure in which cells are randomly oriented towards either the periphery or the central lumen.

42. Use of the organoid of any one of claims 36 to 41 in a drug discovery screen or toxicity assay.

43. Use of the organoid of any one of claims 36 to 41 in the manufacture of a medicament for use in regenerative medicine.

44. Use of epithelial stem cells or isolated tissue fragments comprising said epithelial stem cells in the manufacture of a three dimensional organoid as claimed in any one of claims 37-41 for use in regenerative medicine.