CLAIMS

1. A method for obtaining a liver organoid by expanding epithelial stem cells, wherein said method comprises:

culturing at least one epithelial stem cell from a liver fragment or a liver biliary duct in contact with an extracellular matrix in the presence of a medium, the medium comprising a basal medium for animal or human cells to which is added: Epidermal Growth Factor (EGF), a Wnt agonist, hepatocyte growth factor (HGF) and Nicotinamide.

2. A method according to claim 1, wherein one, two or three from the group: a bone morphogenetic protein (BMP) inhibitor, a TGF-beta inhibitor and a fibroblast growth factor (FGF) are added to the medium.

3. A method according to claim 1 or claim 2, wherein after at least one day, the medium is changed to a second medium.

4. A method according to claim 3, wherein the second medium is a differentiation medium comprising a basal medium for animal or human cells to which is added EGF, FGF and/or HGF, a TGF-beta inhibitor and a Notch inhibitor.

5. A method according to any one of claims 1 to 4, wherein the method comprises culturing said epithelial stem cells in a cell culture medium comprising a basal medium for animal or human cells to which is added EGF, a BMP inhibitor, R-spondin and Wnt; and subsequently in a second expansion medium which comprises a basal medium for animal or human cells to which is added: EGF, an FGF, and HGF, Nicotinamide, and an R-spondin; and subsequently in a differentiation medium which comprises or consists of a basal medium for animal or human cells to which is added: EGF, FGF and/or HGF, a TGF-beta inhibitor, and a Notch inhibitor.

6. A cell culture medium for expanding liver epithelial stem cells to obtain an organoid comprising a basal medium for animal or human cells to which is added: EGF, a Wnt agonist, HGF and Nicotinamide.

7. The cell culture medium according to claim 6, wherein one, two or three from the group: a BMP inhibitor, a TGF-inhibitor and FGF are added to the medium.

8. A method according to any one of claims 1 to 5 or a cell culture medium according to claims 6 or 7 wherein the Wnt agonist is selected from the group consisting of: a Wnt family member, R-spondin 1-4, Norrin, and a GSK-inhibitor.

9. A method according to any one of claims 1 to 5 or a cell culture medium according to claim 7, wherein the TGF-beta inhibitor is a small molecule inhibitor

selected from the group consisting of: A83-01, SB-431542, SB-505124, SB-525334, LY 364947, SD-208, SJN 2511.

10. A method according to any one of claims 2 to 5 or a cell culture medium according to claim 7, wherein the FGF is an FGF able to bind to FGFR2 or FGFR4.

11. A method or a cell culture medium according to claim 10 wherein the FGF is FGF10.

12. A method according to any one of claims 1 to 5 or a cell culture medium according to claim 6 or claim 7, wherein one, two, three, four or more from the group gastrin, B27, N2, and N-Acetylcysteine are added to the basal medium.

13. A population of adult liver stem cells or a liver organoid, wherein the population of cells or liver organoid is obtained by a method of any one of claims 1 to 5 or 8 to 12.

14. A liver organoid obtained by a method of any one of claims 1 to 5 or 8 to 12, which comprises a three dimensional cystic structure and on the outside, a layer of cells with a central lumen.

15. A liver organoid according to claim 13 or claim 14, which has one or more (e.g. 2, 3, 4, 5 or all 6) of the following characteristics:

(a) having a cell density of $>5x10^5$ cells/cm^3,

(b) having a thickness equivalent to 2-30 layers of cells,

(c) the cells mutually contact in three dimensions,

(d) demonstrate a function inherent to healthy liver tissue,

(e) have an elongated shape, with two defined domains,

(f) has at least one bud.

16. A liver organoid according to claim 15 wherein the organoid has a cell density of $>10x10^5$ cells/cm^3.

17. A liver organoid according to claim 15 wherein the organoid has a thickness equivalent to 2-15 layers of cells.

18. A liver organoid according to any one of claims 13 to 17 comprising a single layered epithelial domain where polarized cells are detected and keratin markers are expressed and a second domain constituting the main body of the organoid and formed by a multilayered epithelia with non-polarized cells.

19. A liver organoid according to claim 18 wherein the second domain expresses albumin.

20. A liver organoid according to any one of claims 13 to 19, which originates from a cell expressing Lgr5, and which comprises a population of between at least $1x10^3$ cells and $5x10^4$ cells.

21. A liver organoid according to claim 20 wherein the organoid comprises a population of between at least $1x10^3$ cells and $5x10^3$ cells.

22. Use of a liver organoid as defined in any one of claims 13 to 21 in a drug discovery screen; toxicity assay; research of liver embryology, liver cell lineages, and differentiation pathways; gene expression studies including recombinant gene expression; research of mechanisms involved in liver injury and repair; research of inflammatory and infectious diseases of the liver; studies of pathogenic mechanisms; or studies of mechanisms of liver cell transformation and aetiology of liver cancer.

23. Use of a liver organoid or cell population as defined in any one of claims 13 to 21 in the preparation of a medicament for treating a liver disorder, condition or disease or for use in regenerative medicine.