

Claims

1. A method for expanding cholangiocytes in vitro comprising:
 - (i) providing a population of cholangiocytes and;
 - (ii) culturing the population in the presence of a farnesoid X receptor (FXR) agonist to produce an expanded population of FXR-treated cholangiocytes.
2. A method according to claim 1 wherein the farnesoid X receptor (FXR) agonist is chenodeoxylic acid (CDA) or obeticholic acid (OCA)
3. A method according to claim 1 or claim 2 wherein the population is cultured in an expansion medium comprising the farnesoid X receptor (FXR) agonist, an epidermal growth factor (EGF), a canonical Wnt signalling inhibitor and a non-canonical Wnt signalling potentiator, to produce the expanded population of FXR-treated cholangiocytes.
4. A method according to any one of claims 1 to 3 wherein the cholangiocytes are extrahepatic cholangiocytes, intrahepatic cholangiocytes or gallbladder cholangiocytes.
5. A method according to any one of claims 1 to 4 wherein the non- canonical Wnt signalling potentiator is a Wnt agonist, optionally R-spondin.
6. A method according to any one of claims 1 to 5 wherein the canonical Wnt signalling inhibitor is Dickkopf-related protein 1 (DKK-1).
7. A method according to any one of claims 1 to 7 wherein population is cultured in 3D culture in the expansion medium, optionally wherein the expansion medium further comprises a scaffold matrix, optionally a decellularised human or non-human tissue

extracellular matrix.

8. A method according to any one of claims 1 to 7 wherein the population of cholangiocytes is devoid of stem cells

9. A method according to any one of claims 1 to 8 wherein the cholangiocytes in the expanded population

(i) express CK7, CK18, CK19, HNF1B, Gamma Glutamyl-Transferase (GGT), CFTR, SCR, SSTR2, Apical Salt and Bile Transporter (ASBT), Aquaporin 1 and Anion Exchanger 2, FGF19, SOX17;

(ii) display ALP activity, GGT activity, MDR1 mediated secretion, physiological responses to secretin and somatostatin, export of bile acids, CFTR mediated chloride transfer, physiological responses to ATP and acetylcholine and increased proliferation in response to VEGF; and/or

(iii) do not express MHC antigens or express MHC antigens at levels lower than primary cholangiocytes.

10. A method according to any one of claims 1 to 9 wherein the cholangiocytes are cultured in the expansion medium for 20 or more passages.

11. A method according to any one of claims 1 to 10 comprising seeding the expanded population of FXR-treated cholangiocytes into a biocompatible scaffold, optionally within the scaffold matrix is a decellularised human or non-human tissue extracellular matrix.

12. A method according to claim 11 wherein the cholangiocytes form a biliary epithelium in the scaffold.

13. A method according to any one of claims 1 to 12 comprising storing the expanded population of FXR-treated cholangiocytes or scaffold and/or admixing the expanded population or scaffold with a therapeutically acceptable excipient.

14. An isolated population of FXR-treated cholangiocytes produced by a method of any one of claims 1 to 13.

15. A population according to claim 14 wherein the FXR treated cholangiocytes are within a biocompatible scaffold, optionally wherein the decellularised scaffold matrix is a human or non-human tissue extracellular matrix.

16. A population according to claim 14 or claim 15 wherein the FXR treated cholangiocytes

(i) express CK7, CK18, CK19, HNF1B, Gamma Glutamyl-Transferase (GGT), CFTR, SCR, SSTR2, Apical Salt and Bile Transporter (ASBT), Aquaporin 1 and Anion Exchanger 2, FGF19, SOX17

(ii) do not express MHC antigens or express low levels of MHC antigens, and/or

(iii) display ALP activity, GGT activity, MDR1 mediated secretion, physiological responses to secretin and somatostatin, export of bile acids, CFTR mediated chloride transfer, physiological responses to ATP and acetylcholine and increased proliferation in response to VEGF

17. A biocompatible scaffold comprising an isolated population according to any one claims 14 to 16.

18. Use of an isolated population of FXR-treated cholangiocytes according to any one of claims 14 to 16 or a scaffold according to claim 17 in the manufacture of a medicament for the treatment of a biliary disorder.

19. A method of screening a compound comprising;

contacting a population of isolated FXR treated cholangiocytes according to any one of claims 14 to 16 or a scaffold according to claim 17 with a test compound, and;

determining the effect of the test compound on said the FXR treated cholangiocytes or scaffold and/or the effect of said FXR treated cholangiocytes or scaffold on the test compound,

optionally wherein the effect of the test compound on one or more of proliferation, ALP activity, GGT activity, MDR1 mediated secretion, secretin and/or somatostatin responsiveness, bile acid export, CFTR mediated chloride transfer, and ATP, acetylcholine and VEGF responsiveness is determined.

20. A method of determining the susceptibility of a patient to a therapeutic compound comprising;

(i) providing a population of isolated cholangiocytes having a disease phenotype from an individual with a disease condition;

(ii) culturing the population in an expansion medium comprising an FXR receptor, epidermal growth factor (EGF), a canonical Wnt signalling inhibitor and a non-canonical Wnt signalling potentiator, to produce an expanded population of cholangiocytes displaying a disease phenotype,

(iii) contacting the expanded population of FXR treated cholangiocytes produced by a method described herein with a therapeutic compound, and;

(iv) determining the effect of the therapeutic compound on said FXR treated cholangiocytes,

wherein an amelioration of the disease phenotype of the FXR treated cholangiocytes is indicative that the individual is susceptible to treatment with the therapeutic compound.

21. A kit for production of FXR treated cholangiocyte organoids comprising an expansion

medium comprising an FXR receptor, epidermal growth factor (EGF), a non- canonical Wnt/PCP signalling potentiator and a canonical Wnt signalling inhibitor.

22. Use of a culture medium for the *in vitro* production of FXR treated cholangiocyte organoids, wherein the culture medium comprises an FXR receptor, epidermal growth factor (EGF), a non- canonical Wnt/PCP signalling potentiator and a canonical Wnt signalling inhibitor.