

CLAIMS

1. A method of culturing cells, comprising:
 - (a) providing a microfluidic device comprising a microchannel comprising a surface, the surface covalently attached to a light reactive portion of a N-sulphosuccinimidy-6-(4'-azido-2'-nitrophenylamino) hexanoate (sulfo-SANPAH) crosslinker, the microchannel in fluidic communication with a fluid source comprising fluid;
 - (b) covalently attaching one or more proteins or peptides to a chemically reactive portion of the sulfo-SANPAH crosslinker so as to create a treated surface;
 - (c) seeding viable cells on the treated surface so as to create attached cells;
 - 10 (d) flowing fluid from the fluid source through the microchannel so as to create flowing conditions; and
 - (e) culturing the attached cells under the flow conditions such that the cells remain attached and viable for at least 6 days.
2. A method according to Claim 1, in which the cells are hepatocytes.
- 15 3. A method according to Claim 2, further comprising (f) assessing viability by measuring the level of activity of one or more cellular enzymes.
4. A method according to Claim 3, in which the cellular enzyme is a CYP enzyme.
5. A method according to Claim 3, in which the cellular enzyme is a transaminase.
6. A method according to Claim 1, further comprising (f) assessing viability by measuring
20 the level of expression of one or more cellular proteins.
7. A method according to Claim 1, in which the one or more proteins comprises collagen.
8. A method according to Claim 1, in which the one or more proteins comprises a mixture of collagen type I, fibronectin and collagen type IV.
9. A method according to Claim 1, in which the one or more peptides comprises RGD or a
25 peptide comprising the RGD motif.
10. A method according to Claim 1, in which the attached cells are cultured under flow conditions for 2 days.

11. A method according to Claim 1, in which the attached cells are cultured under flow conditions for 5 days.

12. A method according to Claim 1, in which the attached cells are cultured under flow conditions for 10 days.

5 13. A method according to Claim 12, in which the cells remain attached and viable for at least 14 days.

14. A method of treating a microfluidic device, comprising:

10 (a) providing a microfluidic device comprising a microchannel comprising a surface, the surface covalently attached to a light reactive portion of a N-sulphosuccinimidyl-6-(4'-azido-2'-nitrophenylamino) hexanoate (sulfo-SANPAH) crosslinker, the microchannel in fluidic communication with a fluid source comprising fluid;

(b) covalently attaching one or more proteins or peptides to a chemically reactive portion of the sulfo-SANPAH crosslinker so as to create a treated surface; and

(c) storing the microfluidic device wet and in the absence of cells.

15 15. A method according to Claim 14, in which the storing is done at a controlled temperature below room temperature.

16. A method according to Claim 15, in which the storing is done at between 2 and 10°C.

17. A method according to Claim 14, in which the one or more covalently attached proteins is collagen I.

20 18. A method according to Claim 14, in which the one or more covalently attached proteins is laminin.

19. A method according to Claim 14, in which the method further comprises:

(d) seeding viable cells on the treated surface so as to create attached cells;

25 (e) flowing fluid from the fluid source through the microchannel so as to create flowing conditions; and

(f) culturing the attached cells under the flow conditions such that the cells remain attached and viable for at least 6 days.

20. A method according to Claim 19, in which the cells are hepatocytes.

21. A method according to Claim 19, in which the attached cells are cultured under flow conditions for 2 days.

22. A method according to Claim 19, in which the attached cells are cultured under flow
5 conditions for 5 days.

23. A method according to Claim 19, in which the attached cells are cultured under flow conditions for 10 days.

24. A method according to Claim 23, in which the cells remain attached and viable for at least 14 days.

CLAIMS

1. A method of culturing cells, comprising:
 - (a) providing a microfluidic device comprising a microchannel comprising a surface, the surface covalently attached to a light reactive portion of a N-sulphosuccinimidyl-6-(4'-azido-2'-nitrophenylamino) hexanoate (sulfo-SANPAH) crosslinker, the
 5 microchannel in fluidic communication with a fluid source comprising fluid;
 - (b) covalently attaching one or more extracellular matrix proteins or peptides to a chemically reactive portion of the sulfo-SANPAH crosslinker so as to create a treated surface;
 - 10 (c) seeding viable epithelial cells on the treated surface so as to create attached epithelial cells;
 - (d) flowing fluid from the fluid source through the microchannel so as to create flowing conditions; and
 - (e) culturing the attached epithelial cells under the flow conditions such that the
 15 epithelial cells remain attached and viable for at least 6 days.
2. A method according to Claim 1, in which the epithelial cells are hepatocytes.
3. A method according to Claim 2, further comprising (f) assessing viability by measuring the level of activity of one or more cellular enzymes.
4. A method according to Claim 3, in which the cellular enzyme is a CYP enzyme.
- 20 5. A method according to Claim 3, in which the cellular enzyme is a transaminase.
6. A method according to Claim 1, further comprising (f) assessing viability by measuring the level of expression of one or more cellular proteins.
7. A method according to Claim 1, in which the one or more extracellular matrix proteins comprises collagen.
- 25 8. A method according to Claim 1, in which the one or more extracellular matrix proteins comprises a mixture of collagen type I, fibronectin and collagen type IV.

9. A method according to Claim 1, in which the one or more extracellular matrix peptides comprises RGD or a peptide comprising the RGD motif.
10. A method according to Claim 1, in which the attached epithelial cells are cultured under flow conditions for 10 days.
- 5 11. A method according to Claim 10, in which the epithelial cells remain attached and viable for at least 14 days.
12. A method of treating a microfluidic device, comprising:
 - (a) providing a microfluidic device comprising a microchannel comprising a surface, the surface covalently attached to a light reactive portion of a N-sulphosuccinimidyl-6-
10 (4'-azido-2'-nitrophenylamino) hexanoate (sulfo-SANPAH) crosslinker, the microchannel in fluidic communication with a fluid source comprising fluid;
 - (b) covalently attaching one or more extracellular matrix proteins or peptides to a chemically reactive portion of the sulfo-SANPAH crosslinker so as to create a treated surface;
 - 15 (c) storing the microfluidic device wet and in the absence of cells;
 - (d) seeding viable epithelial cells on the treated surface so as to create attached epithelial cells;
 - (e) flowing fluid from the fluid source through the microchannel so as to create flowing conditions; and
 - 20 (f) culturing the attached epithelial cells under the flow conditions such that the epithelial cells remain attached and viable for at least 6 days.
13. A method according to Claim 12, in which the storing is done at a controlled temperature below room temperature.
14. A method according to Claim 13, in which the storing is done at between 2 and 10°C.
- 25 15. A method according to Claim 12, in which the one or more covalently attached extracellular matrix proteins is collagen I.
16. A method according to Claim 12, in which the one or more covalently attached extracellular matrix proteins is laminin.

2017. A method according to Claim 12, in which the epithelial cells are hepatocytes.

18. A method according to Claim 12, in which the attached epithelial cells are cultured under flow conditions for 10 days.

19. A method according to Claim 18, in which the epithelial cells remain attached and
5 viable for at least 14 days.