Accordingly, described herein is a method for reprogramming human stem cells (e.g. hPSCs) towards the ground state. The method is a method of resetting transcription factor control circuitry towards ground state. As explained below, the methods may also be applied to stem cells from other species.

The resetting/reprogramming methods described herein are demonstrated to reproducibly reset multiple cells lines. Additionally, the inventors have shown that the methods described herein produce stable cells lines having ground state characteristics. Characteristics of the cells produced are described in more details elsewhere herein.

Thus in one aspect, the invention provides methods for resetting cells toward a 'ground' or 'naïve' state. The methods of resetting cells may also be referred to as methods of reprogramming cells. This reprogramming resets transcription factor control circuitry towards ground state pluripotency.

Induction of a naïve state can be done in a first, resetting medium. This naïve state can be sustained, and the cells propagated in a second, naïve culture medium, that comprises a PKC inhibitor.

The present disclosure provides a method of reprogramming a human stem cell to a more naïve state comprising:

a) providing a human stem cell to be reprogrammed,

b) inducing a more naïve state, wherein the inducing comprises optionally expressing or introducing one or more heterologous reprogramming factors into the cell, culturing the in a first medium, wherein the first medium comprises a MEK inhibitor, a WNT pathway inhibitor and preferably a STAT3 activator, and optionally other factors

c) sustaining the cell in a second medium, wherein the second medium comprises the presence of a MEK inhibitor and a PKC inhibitor, and preferably a GSK3 inhibitor and a STAT3 activator, and optionally other factors

d) thereby obtaining a reprogrammed cell.

Thus the present invention provides a method of resetting a human stem cell to a more naïve state, the method comprising: providing a human stem cell to be reset; resetting the transcription factor network, wherein the resetting comprises culturing the cell under a first condition, wherein the first condition is in the presence of a MEK inhibitor and a PKC inhibitor, and sustaining the cell in a second condition, wherein the second condition

CLAIMS

1. A method of resetting a human stem cell to a more naïve state, the method comprising:

(a) providing a human stem cell to be reset,

(b) inducing a more naïve state by

(i) optionally expressing or introducing one or more heterologous reprogramming factors into the cell,

(ii) culturing the cell in a resetting medium, wherein the resetting medium comprises a MEK inhibitor, a WNT pathway inhibitor and optionally a STAT3 activator, and optionally one or more further inhibitors,

(c) sustaining the cell in a naïve culture medium, wherein the naïve culture medium comprises a MEK inhibitor, a PKC inhibitor and optionally a GSK3 inhibitor, and a STAT3 activator.

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2. A method according to claim 1, wherein step (b)(i) is performed to reprogram the cell to a more naïve state and comprises expressing reprogramming factors in the cell, wherein the reprogramming factors comprise or consist of NANOG and KLF2.

3. A method according to claim 2 of reprogramming a human pluripotent stem cell to a more naïve state, the method comprising expressing reprogramming factors in the cell, wherein the reprogramming factors comprise NANOG and KLF2, and culturing the cell in the resetting medium, wherein the resetting medium comprises a MEK inhibitor, and optionally a GSK3 inhibitor and a STAT3 activator or optionally,
a FGF inhibitor and a STAT3 activator.

4. A method according to any one of claims 1 to 3, wherein expression of the reprogramming factors is transient, which is optionally achieved by introducing into the cell a plasmid preparation which expresses the reprogramming factors in the cell
and wherein heterologous nucleic acid encoding the reprogramming factors is not maintained following reprogramming to the naïve state.

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5. A method according to claim 1, wherein step (b)(i) is not performed and the resetting medium further comprises a HDAC inhibitor.

6. A method according to claim 1, wherein step (b)(i) is not performed and the resetting medium further comprises a PKC inhibitor.

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7. A method according to claim 1, wherein the resetting medium comprises a MEK inhibitor, a HDAC inhibitor and a STAT3 activator.

8. A method according to any one of claims 1 to 7, wherein the resetting medium comprises a PKC inhibitor.

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9. A method according to any one of claims 1 to 8, wherein the naïve culture medium comprises a GSK3 inhibitor, a MEK inhibitor and a STAT3 activator and a PKC inhibitor.

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10. A method according to any one of claims 1 to 2, 5 to 6, or 8 to 9, wherein the naive culture medium further comprises a ROCK inhibitor.

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11. A method according to any one of claims 1 to 10 wherein the WNT pathway inhibitor is a tankyrase inhibitor.

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12. A method according to any one of claims 1 to 11,

(i) wherein the GSK3 inhibitor, if present, is CHIR99021; and/or,

(ii) wherein the MEK inhibitor, if present, is PD0325901; and/or,

(iii) wherein the PKC inhibitor, if present, is Gö6983 or Ro-31-8425; and/or,

(iv) wherein the STAT3 activator, if present, is LIF, which is optionally human LIF; and/or

(v) wherein the FGF inhibitor if present, is PD173074; and/or

(vi) wherein the tankyrase or other wnt pathway inhibitor is XAV939 .

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13. A method according to any one of claims 1 to 12, wherein (i) the cells are cultured in an FGF supplemented serum replacement medium prior to inducing the more naïve state, and/or (ii) the resetting medium or naïve culture medium is replaced daily and\or wherein the resetting is performed in the presence of 5% oxygen.

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14. A method according to any one of claims 1 to 13 wherein maintenance in a naïve state is confirmed by one of more of the following phenotypes or genotypes:

a) the ability to continuously and clonally self-renew in culture and retain pluripotency
b) a global transcriptome more similar to that of mouse embryonic stem cells cultured in defined media than to mouse post-implantation epiblast stem cells (EpiSCs) or conventional human pluripotent stem cells
c) a global transcriptome more similar to pre-implantation epiblast than post-implantation epiblast
d) expression of mRNA and protein of pre-implantation epiblast specific transcription factors, optionally 1, 2, 3, 4, 5, 6 or all of: KLF2, KLF4, TFCP2L1, TBX3, REX1, GBX2 and STELLA (DPPA3), and expression of mRNA and protein of general pluripotency factors, such as OCT4, SOX2 and SALL4, and optionally elevated mRNA and protein levels of NANOG;
e) reliance on critical transcription factors defined in mouse embryonic stem cells, particularly TFCP2L1 and KLF4;
f) nuclear localisation of TFE3;
g) low level expression or absence of expression of early lineage markers that are typically expressed in convention human pluripotent stem cells, such as AFP, EOMES and/or BRACHURY;
h) active mitochondrial respiration;
i) genome-wide hypomethylation;
j) lower levels of histone modifications associated with gene , such as reduced levels of H3K27me3 and H3K9me3;
k) capable of incorporation into a host embryo inner cell mass and a pre-implantation epiblast to form embryo chimaeras;
l) able to colonise a post-implantation epiblast and derivative tissues in chimaeras formed with the same, or closely related, species;
m) self-renewal in the presence of complete inhibition of Erk/MAP kinase signalling;
n) self-renewal in the presence of growth factor receptor tyrosine kinase signalling inhibition;
o) self-renewal in the presence of TGFbeta/activin signalling inhibition;
p) self-renewal in the presence of PKC inhibition or knockdown;
q) self-renewal in the presence of partial inhibition of (GSK3) glycogen synthase kinase-3 activity;
r) self-renewal in the presence of STAT3 agonists, such as LIF;

s) self-renewal from dissociated single cells with or without Rho associated kinase inhibition (ROCKi);
t) self-renewal in the absence of serum or serum substitutes;
u) self-renewal in the absence of feeder cells;
v) self-renewal in the absence of transgene expression or other genetic perturbation;
w) retention of diploid karyotype without rearrangement in long-term passaging, for example over more than 40 population doublings;
x) differentiation into conventional primed pluripotent phenotype in the presence of growth factor stimulation of Erk/MAP kinase signalling and activin;
y) able to differentiate in vitro into primordial germ cells as well as somatic germ layers;
z) able to establish continuous culture in vitro by transition from a pre-implantation epiblast;
aa) tightly packed domed appearance;
bb) reduction in expression of DNMT3a and DNMT3b.

15. A method according to claim 14, wherein expression of KLF2, KLF4, TFCP2L1, TBX3, REX1, GBX2 and STELLA is induced in the reprogrammed cells.

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16. A method according to any one of the preceding claims wherein the human cells for use in the method are selected from:
(i) induced pluripotent stem cells;
(ii) cells from an embryonic cell line;
(iii) embryonic stem cells obtained by biopsy without destruction of the respective embryo.

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17. A resetting medium as defined in any one of claims 1 to 13, wherein the resetting medium comprises a MEK inhibitor, a WNT pathway inhibitor and optionally a STAT3 activator, and optionally one or more further inhibitors.

18. A resetting medium according to claim 17, wherein the medium is serum-free.

19. A resetting composition according to claim 17 or claim 18 wherein the WNT pathway inhibitor is a tankyrase inhibitor.