[0007] In order to obtain hESCs which more closely resemble the native pluripotent epiblast, a transgene free method would be advantageous.

[0008] Accordingly, it is an aim of the present invention to provide and maintain a pluripotent stem cell state that ameliorates the disadvantages of the prior art and differentiates more efficiently.

SUMMARY OF THE INVENTION

[0009] In a first aspect there is provided a method for culturing and maintaining a pluripotent stem cell in an undifferentiated state comprising culturing the pluripotent stem cell in a medium comprising an MEK inhibitor, a GSK3 inhibitor, a dual inhibitor of AMPK and/or BMP signaling and LIF.

[0010] In a second aspect there is provided a pluripotent stem cell produced by the method as described herein.

[0011] In a third aspect there is provided a method for generating lineage specific cells from a pluripotent stem cell, comprising: a) culturing a pluripotent stem cell in an undifferentiated state according to the method described herein; b) isolating the undifferentiated pluripotent stem cell; and c) culturing the isolated undifferentiated pluripotent stem cell in a culture medium suitable to differentiate the isolated pluripotent stem cell into a lineage specific cell.

[0012] In a fourth aspect there is provided a kit when used in the method as described herein for culturing and maintaining a pluripotent stem cell in an undifferentiated state comprising a culture medium as described and instructions for use.

[0012a] In a fifth aspect there is provided a culture medium for culturing and maintaining a pluripotent stem cell in an undifferentiated state, wherein said culture medium comprises an MEK inhibitor, GSK3 inhibitor, a dual inhibitor of AMPK and BMP signaling and LIF.

[0012b] In a sixth aspect there is provided a culture medium for culturing and maintaining a pluripotent stem cell in an undifferentiated state, wherein said culture medium comprises an MEK inhibitor, GSK3 inhibitor, a dual inhibitor of AMPK and BMP signaling and LIF.

CLAIMS

1. A method for culturing and maintaining a pluripotent stem cell in an undifferentiated state comprising culturing the pluripotent stem cell in a medium comprising an MEK inhibitor, a GSK3 inhibitor, a dual inhibitor of AMPK and BMP signaling and LIF.

2. The method of claim 1, wherein the medium is a conditioned medium.

3. The method of claim 1, wherein the medium comprises feeder cells.

4. The method of claim 1, wherein the medium is free of feeder cells.

5. The method of claim 1, wherein the pluripotent stem cell is cultured on a matrix.

6. The method of claim 5, wherein the matrix is a basement membrane matrix.

7. The method of claim 5, wherein the matrix is an extracellular matrix comprising one or more of laminin, collagen, gelatin, fibronectin, proteoglycan, entactin, heparan sulfate, or synthetic biopolymers.

8. The method of claim 1, wherein the pluripotent stem cell is an embryonic stem cell.

9. The method of claim 8, wherein the embryonic stem cell is human.

10. The method of claim 1, further comprising passaging the pluripotent stem cell in an undifferentiated state.

11. The method of any one of claims 1–10, wherein the MEK inhibitor is in a concentration of 0.5–1μM.

12. The method of any one of claims 1–11, wherein the GSK3 inhibitor is at a concentration of 1–2μM.

13. The method of any one of claims 1–12, wherein the dual inhibitor of AMPK and BMP signaling is at a concentration of 2μM.

14. The method of any one of claims 1–13, wherein the LIF is at a concentration of 8–10ng/ml.

15. A pluripotent stem cell produced by the method of any one of claims 1–14.

16. The pluripotent stem cell of claim 15, wherein the cell expresses at least one pluripotency marker selected from the group consisting of NANOG, TRA-1-60, OCT4 and TRA-1-81.

17. The pluripotent stem cell of claim 15, wherein the cell expresses at least one epiblast-like gene expression marker selected from the group consisting of DPPA3, KLF4, KLF5, and TBX3.

18. The pluripotent stem cell of claim 15, wherein the cell expresses at least one extra-embryonic marker selected from the group consisting of CDX2, BMP4, GATA4 and GATA6.

19. The pluripotent stem cell of claim 15, wherein the cell expresses at least one mesendoderm marker selected from T or MIXL1.

20. The pluripotent stem cell of any one of claims 15–19, wherein the expression level of at least one marker is increased relative to a cell that has not been cultured in accordance with the method of any one of claims 1–14.

21. The pluripotent stem cell of claim 20, wherein the expression level of the at least one marker is determined by RNA–seq or real–time PCR.

22. The pluripotent stem cell of any one of claims 20 to 21, wherein the expression level of the at least one marker is increased by at least 1.5 to 5 fold relative to a cell that has not been cultured in accordance with the method of any one of claims 1–14.

23. The pluripotent stem cell of any one of claims 20 to 22, wherein the at least one marker is NANOG and the expression level is increased by 1.5 to 2 fold relative to a cell that has not been cultured in accordance with the method of any one of claims 1–14.

24. The pluripotent stem cell of claim 15–19, wherein the differentiation of said cell into an endoderm, a neuronal or a mesoderm cell is enhanced relative to differentiation of a cell that has not been cultured in accordance with the method of any one of claims 1–14.

25. A culture medium for culturing and maintaining a pluripotent stem cell in an undifferentiated state, wherein said culture medium comprises an MEK inhibitor, GSK3 inhibitor, a dual inhibitor of AMPK and BMP signaling and LIF.

26. A method for generating lineage specific cells from a pluripotent stem cell, comprising:

a) culturing a pluripotent stem cell in an undifferentiated state according to the method of any one of claims 1–14;

b) isolating the undifferentiated pluripotent stem cell; and

c) culturing the isolated undifferentiated pluripotent stem cell in a culture medium suitable to differentiate the isolated pluripotent stem cell into a lineage specific cell.

27. The method of claim 26, wherein the lineage specific cells are somatic cells or organoids.

28. The method of claim 27, wherein the lineage specific cells are endoderm lineage cells.

29. The method of claim 27, wherein the somatic cell is a committed progenitor cell capable of self-renewal or differentiation into one or several somatic lineages, or a fully mature somatic differentiated cell.

30. A kit when used in the method of any one of claims 1–14 for culturing and maintaining a pluripotent stem cell in an undifferentiated state comprising a culture medium according to claim 24 and instructions for use.