

towards a pluripotent state for cell therapy. Until now there is a deficiency in industrial processes for automated large-scale generation and expansion of iPSCs.

[0008] Thus, there is a need for a method of reprogramming somatic cell into an undifferentiated cell, such as iPSCs, that addresses, or at least ameliorates, one or more of the disadvantages described above. A high-throughput method that enables the large-scale production of iPSCs holds great promise for revolutionizing regenerative medicine.

SUMMARY

[0009] In one example, the present disclosure refers to a method of reprogramming somatic cells into induced pluripotent stem cells (iPSCs), comprising:

- (a) seeding the somatic cells on a plurality of microcarriers (MCs) to form cell-MC aggregates; and
- (b) transducing transcription factors into the somatic cells of the cell-MC aggregates; wherein step (a) and step (b) are carried out under continuous agitation.

[0009a] In one aspect, the present disclosure refers to a method of reprogramming somatic cells into induced pluripotent stem cells (iPSCs), comprising: (a) seeding the somatic cells on a plurality of microcarriers (MCs) to form cell-MC aggregates; (b) transducing transcription factors into the somatic cells of the cell-MC aggregates; and (c) immobilizing the cell-MC aggregates into a hydrogel; wherein step (a) and step (b) are carried out under continuous agitation; wherein the transcription factors comprise Oct4, Sox2 and Klf4, wherein the somatic cells are fibroblast cells, wherein the MCs are positively-charged polystyrene MCs, and wherein the MCs are coated by extracellular matrix (ECM).

[0010] In one example, the present disclosure refers to a method of producing, selecting, expanding, characterizing, and differentiating iPSCs, comprising:

- carrying out steps (a)-(b) of the first aspect;
- (c) immobilizing the cell-MC aggregates into a hydrogel;
- (d) selecting the cell-MC aggregates showing fast cell growth or expressing pluripotency markers;
- (e) expanding the selected cell-MC aggregates by adding fresh MCs to the selected cell-MC aggregates;
- (f) characterising the cell-MC aggregates to determine whether the somatic cells on the MCs have been reprogrammed to iPSCs; and
- (g) differentiating the cells of the cell-MC aggregates toward functional cells.

[0010a] In one aspect, the present disclosure refers to a method of producing, selecting, expanding, characterizing, and differentiating iPSCs, comprising: carrying out steps (a)-(c) disclosed herein; (d) selecting the cell-MC aggregates showing an increase in size of at least 2 times within 7 days compared to the size of the cell-MC aggregates immobilized in step (c) disclosed herein or expressing pluripotency markers; (e) expanding the selected cell-MC aggregates by adding fresh MCs to the selected cell-MC aggregates; (f) characterising the cell-MC aggregates to determine whether the somatic cells on the MCs have been reprogrammed to iPSCs; and (g) differentiating the cells of the cell-MC aggregates toward functional cells, wherein the MCs of step (a) disclosed herein are coated by extracellular matrix (ECM).

[0011] In one example, the present disclosure refers to a method of reprogramming somatic cells selected from the group consisting of fibroblasts IMR90, fibroblasts HFF-01, PBMC, CD3⁺ T cells and CD34⁺ hematopoietic stem cells (HSCs) into induced pluripotent stem cells (iPSCs), comprising:

- (a) seeding the somatic cells on a plurality of microcarriers (MCs) to form cell-MC aggregates;

- (b) transducing transcription factors into the somatic cells of the cell-MC aggregates using Sendai virus;
- (c) immobilizing the cell-MC aggregates into a hydrogel;
- (d) selecting the cell-MC aggregates showing fast cell growth or expressing pluripotency markers;
- (e) expanding the selected cell-MC aggregates by adding fresh MCs to the selected cell-MC aggregates; and
- (f) characterising the cell-MC aggregates to determine whether the somatic cells on the MCs have been reprogrammed to iPSCs;

wherein step (a) and step (b) are carried out under continuous agitation; and

wherein the transcription factors comprise Oct4, Sox2, c-Myc, and Klf4.

[0011a] In one aspect, the present disclosure refers to a method of reprogramming somatic cells selected from the group consisting of fibroblasts IMR90 and fibroblasts HFF-01 into induced pluripotent stem cells (iPSCs), comprising: (a) seeding the somatic cells on a plurality of microcarriers (MCs) to form cell-MC aggregates; (b) transducing transcription factors into the somatic cells of the cell-MC aggregates using Sendai virus; (c) immobilizing the cell-MC aggregates into a hydrogel; (d) selecting the cell-MC aggregates showing an increase in size of at least 2 times within 7 days compared to the size of the cell-MC aggregates immobilized in step (c) or expressing pluripotency markers; (e) expanding the selected cell-MC aggregates by adding fresh MCs to the selected cell-MC aggregates; and (f) characterising the cell-MC aggregates to determine whether the somatic cells on the MCs have been reprogrammed to iPSCs; wherein step (a) and step (b) are carried out under continuous agitation; wherein the transcription factors comprise Oct4, Sox2, c-Myc, and Klf4, wherein the MCs are positively-charged polystyrene MCs, and wherein the MCs are coated by extracellular matrix (ECM).

BRIEF DESCRIPTION OF DRAWINGS

[0012] The present disclosure will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0013] FIG. 1, comprising FIGs. 1(A) and 1(B), is a schematic diagram for somatic cell reprogramming, wherein FIG. 1(A) shows conventional monolayer (MNL) approach, and FIG. 1(B) shows ReprograMC approaches (Method A and Method B).

[0014] FIG. 2 shows the morphology of derived-iPSCs colonies reprogrammed on monolayer cultures 2 hours and on Day 7 post transduction, on Day 15 (when manually picked), on Day 23 (when expanded on laminin-coated tissue culture plates), and on Day 31 (when frozen down for banking). Scale bars, 200µm.

[0015] FIG. 3, comprising FIGs. 3(A) and 3(B), shows the characterization of iPSC clones derived from fibroblasts on monolayer cultures, wherein FIG. 3(A) shows high expression of the pluripotent markers Tra-1-60 and Oct4 detected by FACS analysis, and FIG. 3(B) shows immunochemical staining of pluripotency marker (Tra-1-60) which further confirms the pluripotency of iPSC clones. Scale bars, 500µm

[0016] FIG. 4, comprising FIGs. 4(A), 4(B) and 4(C), shows the monitoring, screening and selection of transduced cell-MC aggregates, wherein FIG. 4(A) shows the representative images of transduced IMR90 cell attachment, embedded hydrogel cultures, and cell-MC selection with Tra-1-60 pluripotent antibody marker, FIG. 4(B) shows the representative

CLAIMS

1. A method of reprogramming somatic cells into induced pluripotent stem cells (iPSCs), comprising:

(a) seeding the somatic cells on a plurality of microcarriers (MCs) to form cell-MC aggregates;

(b) transducing transcription factors into the somatic cells of the cell-MC aggregates; and

(c) immobilizing the cell-MC aggregates into a hydrogel;

wherein step (a) and step (b) are carried out under continuous agitation;

wherein the transcription factors comprise Oct4, Sox2 and Klf4,

wherein the somatic cells are fibroblast cells,

wherein the MCs are positively-charged polystyrene MCs, and

wherein the MCs are coated by extracellular matrix (ECM).

2. The method of claim 1, wherein the transcription factors consist of Oct4, Sox2 and Klf4.

3. The method of claim 1, wherein the transcription factors comprise Oct4, Sox2 and Klf4 and exclude c-Myc.

4. The method of claim 1, wherein the transcription factors further comprise one or more of Nanog, c-Myc, and LIN28.

5. The method of claim 3, wherein the transcription factors further comprise one or more of Nanog and LIN28.

6. The method of any one of the preceding claims, wherein the transduction is done via an agent selected from the group consisting of virus, protein, plasmids, and PiggyBac.

7. The method of claim 6, wherein the protein is an Embryonic Stem Cell (ESC)-derived extract protein, or a Cell-Penetrating Peptide selected from the group consisting of a designed peptide, a natural protein-derived peptide, and a chimeric peptide.

8. The method of claim 6, wherein the plasmid is an expression plasmid comprising the complementary DNAs (cDNAs) of Oct3/4, Sox2, and Klf4.

9. The method of claim 6, wherein the virus is selected from a group consisting of respirovirus, lentivirus, retrovirus, and adenovirus.

10. The method of claim 9, wherein the respirovirus is Sendai virus.

11. The method of any one of the preceding claims, wherein the fibroblast cells are selected from the group consisting of human foreskin fibroblasts (HFF), human dermal fibroblasts and human lung fibroblasts.

12. The method of any one of the preceding claims, wherein the fibroblast cells are human lung fibroblasts.

13. The method of any one of the preceding claims, wherein the ECM is selected from the group consisting of laminin, vitronectin, fibronectin, heparan sulfate, and collagen.

14. The method of any one of the preceding claims, wherein the size of the MCs is 90-200 μm .

15. The method of any one of claims 1-14, further comprising:

(d) selecting the cell-MC aggregates showing an increase in size of at least 2 times within 7 days compared to the size of the cell-MC aggregates immobilized in step (c) of claim 1 or expressing pluripotency markers;

(e) expanding the selected cell-MC aggregates by adding fresh MCs to the selected cell-MC aggregates; and

(f) characterising the cell-MC aggregates to determine whether the somatic cells on the MCs have been reprogrammed to iPSCs.

16. A method of producing, selecting, expanding, characterizing, and differentiating iPSCs, comprising:

carrying out steps (a)-(c) of claim 1;

(d) selecting the cell-MC aggregates showing an increase in size of at least 2 times within 7 days compared to the size of the cell-MC aggregates immobilized in step (c) of claim 1 or expressing pluripotency markers;

(e) expanding the selected cell-MC aggregates by adding fresh MCs to the selected cell-MC aggregates;

(f) characterising the cell-MC aggregates to determine whether the somatic cells on the MCs have been reprogrammed to iPSCs; and

(g) differentiating the cells of the cell-MC aggregates toward functional cells,

wherein the MCs of step (a) of claim 1 are coated by extracellular matrix (ECM).

17. The method of claim 15 or 16, wherein the hydrogel is an agarose gel.

18. The method of any one of claims 15-17, wherein the pluripotency marker in step (d) is selected from the group consisting of Tra-1-60 and Tra-1-81.

5 19. The method of any one of claims 15-18, wherein the characterization in step (f) is selected from the group consisting of FACS analysis for pluripotency, evaluation of cell growth capacity, evaluation of multi-passage stability, karyotyping, and evaluation of *in vitro* tri-lineage differentiation.

10 20. The method of any one of claims 16-19, wherein the differentiation in step (g) is via formation of Embryoid bodies (EBs)–like cell-MC aggregates.

21. The method of claim 20, wherein the formation of EBs-like cell-MC aggregates is by continuous agitation.

22. The method of any one of claims 15-21, wherein the step (e) is performed under continuous agitation.

15 23. The method of claim 22, wherein the continuous agitation occurs in a stirred bioreactor system.

24. The method of claim 23, wherein the stirred bioreactor system comprises controlled dissolved oxygen, temperature, and pH.

20 25. The method of any one of the preceding claims, wherein the steps are integrated with an automated machine.

26. A method of reprogramming somatic cells selected from the group consisting of fibroblasts IMR90 and fibroblasts HFF-01 into induced pluripotent stem cells (iPSCs), comprising:

(a) seeding the somatic cells on a plurality of microcarriers (MCs) to form cell-MC aggregates;

25 (b) transducing transcription factors into the somatic cells of the cell-MC aggregates using Sendai virus;

(c) immobilizing the cell-MC aggregates into a hydrogel;

(d) selecting the cell-MC aggregates showing an increase in size of at least 2 times within 7 days compared to the size of the cell-MC aggregates immobilized in step (c) or expressing pluripotency markers;

5 (e) expanding the selected cell-MC aggregates by adding fresh MCs to the selected cell-MC aggregates; and

(f) characterising the cell-MC aggregates to determine whether the somatic cells on the MCs have been reprogrammed to iPSCs;

wherein step (a) and step (b) are carried out under continuous agitation;

wherein the transcription factors comprise Oct4, Sox2, c-Myc, and Klf4,

10 wherein the MCs are positively-charged polystyrene MCs, and

wherein the MCs are coated by extracellular matrix (ECM).