[0006] Blood is considered an ideal source of cells for reprogramming due to its abundance and accessibility. Progenitor blood cells from mobilized, bone-marrow and cord blood are highly amenable to reprogramming (Loh et al., 2009; Ye et al., 2009; Giorgetti et al., 2009; Haase et al., 2009). However, cytokine-induced mobilization may result in side effects (Sohn et al., 2002), bone-marrow harvesting is invasive, and cord blood is limited to individuals who have deposited their samples at birth.

[0007] In comparison, peripheral blood T-cells can be easily obtained from donors and reprogrammed with high efficiency (Loh et al., 2010; Seki et al., 2010; Staerk et al., 2010). However, T-cell derived hiPSCs carry pre-existing V(D)J rearrangement at the T-cell receptor (TCR) loci and it is unclear whether these hiPSCs can differentiate normally or if such cells demonstrate tendencies for development into T-cell lymphomas (Serwold et al., 2007).

[0008] Recent studies report the reprogramming of non T-cell populations in peripheral blood. The reprogramming efficiencies in these studies are generally low, necessitating the addition of more factors (5-7 factors in some cases, including SV40LT, *TP53* shRNA) in the reprogramming protocols (Chou et al., 2011; Mack et al., 2011; Okita et al., 2013).

SUMMARY

[0009] The present disclosure relates to methods for obtaining iPSCs from a small volume of peripheral blood.

[0010] The methods require only small blood volumes, for example as little as 10 μl of blood, due to the exclusion of polysaccharide preparations that are typically used to separate blood samples into various components.

[0011] Thus, the blood sample may be obtained by finger-prick. Finger-prick samples can be easily and conveniently collected without the need for trained phlebotomists, including by the subject at home or in another non-laboratory setting.

[0012] The use of Sendai virus to transfect CD71+ cells with pluripotency protein factors can provide iPSCs that are transgene free and that do not contain genomic rearrangement.

[0013] The methods may provide efficient rates of inducing pluripotency in an expanded finger-prick blood cell population. For example, using the method, finger-prick derived hiPSCs were generated from human peripheral blood at very high efficiency (100-600 colonies/mL of blood).

[0014] The ease of obtaining blood samples and the efficient rate of inducing pluripotency may allow for development of international hiPSC banking from large cohorts of donors.

[0015] Disclosed is a method of inducing pluripotency in a hematopoietic cell, the method comprising: providing a blood sample collected from a subject in the absence of any polysaccharide preparation used for separating blood components; depleting the sample of red blood cells by treating the sample with a hypotonic erythrocyte lysis buffer in the absence of any polysaccharide preparation used for separating blood components, to obtain a remaining cell population; expanding the remaining cell population in the sample in a hematopoietic expansion medium to obtain an expanded cell population that contains CD71+ cells; and culturing the expanded cell population in the presence of Oct4, Sox2, and Klf4, and optionally c-MYC, in human embryonic stem cell medium to induce pluripotency.

[0016] The sample may be stored for up to about 48 hours prior to use in the method, at a temperature of about 3°C to about 5°C or on ice.

[0017] The volume of the blood sample may be about 250 µl or less, including for example, from about 10 µl to about 20 µl.

[0018] Disclosed is a method of inducing pluripotency in a hematopoietic cell, the method comprising: providing a blood sample collected from a subject in the absence of any polysaccharide preparation used for separating blood components, the blood sample having a volume of from about 10 µl to about 20 µl and being a sample previously collected by the subject by finger prick in a non-laboratory setting and which has been stored for up to about 48 hours, at a

temperature of about 3°C to about 5°C or on ice; depleting the sample of red blood cells by treating the sample with a hypotonic erythrocyte lysis buffer in the absence of any polysaccharide preparation used for separating blood components, to obtain a remaining cell population; expanding the remaining cell population in the sample in a hematopoietic expansion medium to obtain an expanded cell population that contains CD71+ cells; and culturing the expanded cell population in the presence of Oct4, Sox2, and Klf4, and optionally c-MYC, in human embryonic stem cell medium to induce pluripotency.

[0018a] In one aspect, there is provided a method of inducing pluripotency in a hematopoietic cell, the method comprising:

providing 250 µl or less of a blood sample collected from a subject in the absence of any polysaccharide preparation used for separating blood components;

depleting the sample of red blood cells by treating the sample with a hypotonic erythrocyte lysis buffer in the absence of any polysaccharide preparation used for separating blood components, to obtain a remaining cell population that contains CD71+ cells;

expanding the remaining cell population in the sample in a hematopoietic expansion medium to obtain an expanded cell population that contains 50 % or greater CD71+ cells; and

culturing the expanded cell population in the presence of Oct4, Sox2, and Klf4, and optionally c-MYC, in human embryonic stem cell medium to induce pluripotency.

[0018b] In another aspect, there is provided a method of inducing pluripotency in a hematopoietic cell, the method comprising:

providing a blood sample collected from a subject in the absence of any polysaccharide preparation used for separating blood components, the blood sample having a volume of from 10 µl to 20 µl and being a sample previously collected by the subject by finger prick in a non-laboratory setting and which has been stored for up to 48 hours, at a temperature of 3°C to 5°C or on ice;

depleting the sample of red blood cells by treating the sample with a hypotonic erythrocyte lysis buffer in the absence of any polysaccharide preparation used for separating blood components, to obtain a remaining cell population that contains CD71+ cells;

expanding the remaining cell population in the sample in a hematopoietic expansion medium to obtain an expanded cell population that contains 50 % or greater CD71+ cells; and

culturing the expanded cell population in the presence of Oct4, Sox2, and Klf4, and optionally c-MYC, in human embryonic stem cell medium to induce pluripotency.

[0019] As described herein, the hematopoietic cell may be a non-human hematopoietic cell, or may be a human hematopoietic cell.

[0020] The hypotonic lysis buffer may comprise ammonium chloride.

[0021] Expanding of the remaining cell population in the sample may be performed in a serum-free hematopoietic expansion medium. As well, the expanding may occur for a period of about 12 to about 16 days.

[0022] The resulting expanded cell population may, prior to the culturing to induce pluripotency, comprise about 20 000 cells or greater. The expanded cell population may, prior to the culturing to induce pluripotency, comprise about 50 % or greater CD71+ cells.

[0023] Culturing to induce pluripotency may be performed in the presence or absence of feeder cells. In some embodiments, the Oct4, Sox2 and Klf4, and the optional c-MYC, may be included in the human embryonic stem cell medium during the culturing. In some embodiments, the expanded cell population may be transfected or transduced the with one or more nucleic acid vectors encoding the Oct4, Sox2 and Klf4, and the optional c-MYC during the culturing. For example, the one or more nucleic acid vectors may be viral vectors, episomal vectors, minicircle vectors, or synthesized mRNA. Viral vectors that may be used include adenoviral vectors, baculoviral vectors or Sendai viral vectors. In some embodiments, Sendai viruses are used to transduce the expanded cell population, and, for example, the expanded cell population may be exposed to the one or more Sendai viruses over a period of about 24 hours, including for example at an MOI of about 10 for each of the one or more Sendai viruses.

[0024] Once obtained, the resulting induced pluripotent cells may be stored in a cell bank or library.

[0025] Additionally, a portion of the originally collected blood sample may be, prior to the depleting, reserved for characterization, for example DNA sequencing and/or blood serotyping.

[0026] Disclosed is a kit for collecting a finger prick blood sample for use in inducing pluripotency in a hematopoietic cell, the kit comprising a finger-prick device, a collection tube, a thermo-box, and instructions for collecting the blood sample. The kit may also include a shipping box or packaging for delivering the sample once collected, and/or one or more blood analysis tests.

[0026a] In another aspect, there is provided use of a kit in the collection of a finger prick blood sample according to a method as described herein, wherein the blood sample is free from a polysaccharide preparation, the kit comprising a finger-prick device, a collection tube, a thermo-box, and instructions for collecting the blood sample.

[0027] Other aspects and features of the present disclosure will become apparent to those of ordinary skill in the art upon review of the following description of specific embodiments of the disclosure in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] The figures, which illustrate, by way of example only, embodiments of the present invention, are as follows.

[0029] Figure 1. Derivation of hiPSCs from finger-prick blood samples. (A) Schematic drawing of the strategy used for reprogramming human finger-pricked blood. (B) Immuno-fluorescent staining of FPiPSC colonies expressing pluripotency markers for TRA-1-60, SSEA4 and OCT4. (C) Bisulfite DNA methylation analysis on the promoter of OCT4 and NANOG in FPiPSCs and in their parental cells. (D) Dendrogram showing un-supervised hierarchical clustering of the global expression profiles of hESCs, hiPSCs and parental blood cells (Top). Heat-map analysis of the hESCs, hiPSCs and parental blood cells for expression of Pluripotency and Hematopoietic specific marker genes (Bottom). (E) Scatter plots comparing FPiPSCs global gene expression profiles to parental cells (left) and H7 human ESCs (right). (F) qPvT-PCR analyses for the expression of ESC marker genes OCT4, SOX2, NANOG, LIN28, DNMT3B, REX1, KLF4 and c-MYC in FPiPSCs, H7 ESCs and parental cells.

WHAT IS CLAIMED IS:

1. A method of inducing pluripotency in a hematopoietic cell, the method comprising:

providing 250 µl or less of a blood sample collected from a subject in the absence of any polysaccharide preparation used for separating blood components;

depleting the sample of red blood cells by treating the sample with a hypotonic erythrocyte lysis buffer in the absence of any polysaccharide preparation used for separating blood components, to obtain a remaining cell population that contains CD71+ cells;

expanding the remaining cell population in the sample in a hematopoietic expansion medium to obtain an expanded cell population that contains 50 % or greater CD71+ cells; and

culturing the expanded cell population in the presence of Oct4, Sox2, and Klf4, and optionally c-MYC, in human embryonic stem cell medium to induce pluripotency.

2. The method of any one of claims 1 to 4, wherein the sample has been stored for up to 48 hours prior to use in the method, at a temperature of 3°C to 5°C or on ice.

3. The method of claim 1 or 2, wherein said providing comprises providing from 10 µl to 20 µl of the blood sample.

4. A method of inducing pluripotency in a hematopoietic cell, the method comprising:

providing a blood sample collected from a subject in the absence of any polysaccharide preparation used for separating blood components, the blood sample having a volume of from 10 µl to 20 µl and being a sample previously collected by the subject by finger prick in a non-laboratory setting and which has been stored for up to 48 hours, at a temperature of 3°C to 5°C or on ice;

depleting the sample of red blood cells by treating the sample with a hypotonic erythrocyte lysis buffer in the absence of any polysaccharide preparation used for separating blood components, to obtain a remaining cell population that contains CD71+ cells;

expanding the remaining cell population in the sample in a hematopoietic expansion medium to obtain an expanded cell population that contains 50 % or greater CD71+ cells; and

culturing the expanded cell population in the presence of Oct4, Sox2, and Klf4, and optionally c-MYC, in human embryonic stem cell medium to induce pluripotency.

5. The method of any one of claims 1 to 4, wherein the hematopoietic cell is a non-human hematopoietic cell.

6. The method of any one of claims 1 to 4, wherein the hematopoietic cell is a human hematopoietic cell.

7. The method of any one of claims 1 to 6, wherein the hypotonic lysis buffer comprises ammonium chloride.

8. The method of any one of claims 1 to 7, wherein the expanding is performed in a serum-free hematopoietic expansion medium.

9. The method of any one of claims 1 to 8, wherein the expanding occurs for a period of 12 to 16 days.

10. The method of any one of claims 1 to 9, wherein the expanded cell population, prior to the culturing to induce pluripotency, comprises 20 000 cells or greater.

11. The method of any one of claims 1 to 10, wherein the culturing to induce pluripotency is performed in the presence of feeder cells.

12. The method of any one of claims 1 to 11, wherein the culturing to induce pluripotency comprises including the Oct4, Sox2 and Klf4, and the optional c-MYC, in the human embryonic stem cell medium.

13. The method of any one of claims 1 to 12, wherein the culturing to induce pluripotency comprises transfecting or transducing the expanded cell population with one or more nucleic acid vectors encoding the Oct4, Sox2 and Klf4, and the optional c-MYC.

14. The method of claim 13, wherein the one or more nucleic acid vectors are viral vectors, episomal vectors, minicircle vectors, or synthesized mRNA.

15. The method of claim 14, wherein the one or more nucleic acid vectors are viral vectors that are adenoviral vectors, baculoviral vectors or Sendai viral vectors.

16. The method of claim 14, wherein the one or more viral vectors are Sendai viral vectors.

17. The method of claim 16, wherein the transducing comprises exposing the expanded cell population to the one or more Sendai viral vectors over a period of 24 hours.

18. The method of claim 16 or 17, wherein transducing comprises transducing with an MOI of 10 for each of the one or more Sendai viruses.

19. The method of any one of claims 1 to 18, further comprising storing the resulting induced pluripotent cells in a cell bank or library.

20. The method of any one of claims 1 to 19, wherein a portion of the blood sample is, prior to the depleting, reserved for characterization, for example DNA sequencing and/or blood serotyping.

21. The method of any one of claims 1 to 20, further comprising collecting the blood sample via finger prick prior to said providing.

22. Use of a kit in the collection of a finger prick blood sample according to the method of claim 21, wherein the blood sample is free from a polysaccharide preparation, the kit comprising a finger-prick device, a collection tube, a thermo-box, and instructions for collecting the blood sample.

23. The use of claim 22 further comprising a shipping box or packaging for delivering the sample once collected.

24. The use of claim 22 or 23 further comprising one or more blood analysis tests.