[0023] These pluripotent stem cells can be maintained for somewhat long term indefinite periods in culture, making them a potential source for biologic manufacturing such as for recombinant proteins, DNA, and viruses.

Induced or Reprogrammed spPSC

[0024] Induced Pluripotent Stem Cells are similar to natural pluripotent stem cells, such as embryonic stem (ES) cells, in many respects, such as the expression of certain stem cell genes and proteins, chromatin methylation patterns, doubling time, embryoid body formation, teratoma formation, viable chimera formation, and potency and differentiability.

[0025] Induced pluripotent stem cells, commonly abbreviated as iPS cells or iPSCs are a type of pluripotent stem cell artificially derived from a non-pluripotent cell, typically an adult somatic cell, by inducing a "forced" expression of specific genes (*Nature Reports Stem Cells*. 2007).

[0026] Induced Pluripotent Stem Cells (iPSCs) are produced by transfection of certain stem-cell associated genes into non-pluripotent cells. Induced pluripotent stem cells are typically derived by transfection of certain stem cell-associated genes into non-pluripotent cells such as adult fibroblasts. Transfection is typically achieved through viral vectors, such as retroviruses or retrotransposons. Transfected genes include the master transcriptional regulators Oct-3/4 (Pou5f1) and Sox2, although it is suggested that other genes enhance the efficiency of induction. After 3–4 weeks, small numbers of transfected cells begin to become morphologically and biochemically similar to pluripotent stem cells, and are typically isolated through morphological selection, doubling time, or through a reporter gene and antibiotic selection.

[0027] Embryonic stem cell derived fibroblasts and adult fibroblasts and other cells have been reprogrammed to a pluripotent state by fusion with embryonic stem cells (*Cell. 126*:652-655, 2006 and *Stem Cell Rev, 2*: 331-340, 2006), by the addition of 4 genes using retroviral transfection techniques (*Cell. 126*:663-676, 2006), by a single cassette or bicistronic lentiviral transfection methods [*Stem Cells., 27*:543-549, 2009, and *Stem Cells.* , *27*:1042-1049, 2009), and by endogenous stimulation of pluripotency required transcription factors (*Stem Cells.* , *27*:3053-3062, 2009). Induction or maintenance of pluripotency could also be achieved by modifying methylation or polyadenylation status of the genome (*PLoS One., 4*:e8419, 2009), by microRNAs (*Dev Biol., 344*:16-25, 2010), small molecule activators of required transcription factors, epigenetic reprogramming (*Regen Med., 2*:795-816, 2007), by protein-based reprogramming, (*Blood 116*: 386-395, 2010) by addition of culture supernatant or cell extracts from pluripotent cells in culture, by chemical or radiation or other means of genetic

GeneChip analysis, the authors demonstrated that the reprogrammed neural progenitor cells retained some expression of neural stem cell genes. Polo et.al. (*Nat Biotechnol*, 28, : 848-855, 2010), derived pluripotent reprogrammed cells from mouse tail tip derived fibroblasts, splenic B cells, bone marrow granulocytes and skeletal muscle precursors. Autonomous differentiation studies indicated that splenic B cell and bone marrow granulocyte reprogrammed spPSCs cells gave rise to hematopoietic progenitors more efficiently than fibroblast or skeletal muscle derived spPSCs cells. Interestingly, serial passage of these various spPSCs cell lines led to the abrogation of genetic and methylation differences by passage 16, and the cells also then demonstrated equivalent differentiation efficiencies, in contrast to the earlier results at passage 4. Interestingly, this phenomenon of differential differentiation potential is not restricted to reprogrammed somatic cells, but has also been observed for embryonic stem cells lines, which have been found to have differing genetic signatures and spontaneous preference for differentiation towards certain cell lineages (*Nat Biotechnol.* , 26: 313-315, 2008) (*Hum Reprod Update.* , 13: 103-120, 2007) (*Dev Biol.* , 307:446-459, 2007) (*BMC Cell Biol*, 10:44, 2009) .

[0060] Stem cells can be characterized and isolated by specific antigens expressed on their surface. Pluripotent stem cells can be characterized by the expression of stage-specific embryonic antigen (SSEA), the transcription factors Oct4 and Nanog and other markers, among other methods. Embryonic stem cell derived fibroblasts and adult fibroblasts and other cells have been reprogrammed to a pluripotent state by fusion with embryonic stem cells (*Cell* 126: 652-655, 2006 and *Stem Cell Rev*, 2: 331-340, 2006), by the addition of 4 genes using retroviral transfection techniques (*Cell* 126: 663-676, 2006) , by a single cassette or bicistronic lentiviral transfection methods (*Stem Cells* 27: 543-549, 2009 and *Stem Cells* 27: 1042-1049, 2009), and by endogenous stimulation of pluripotency required transcription factors (*Stem Cells* 27: 3053-3062, 2009) . Induction or maintenance of pluripotency could also be achieved by modifying methylation or polyadenylation status of the genome (*PLoS One* 4: e8419, 2009) , by microRNAs (*Dev Biol.* 344: 16-25, 2010), small molecule activators of required transcription factors, epigenetic reprogramming (*Regen Med.* 2: 795-816, 2007), by protein-based reprogramming (*Blood* 116: 386-395, 2010), by addition of culture supernatant or cell extracts from pluripotent cells in culture, chemical or radiation or other means of genetic mutation to reactivate pluripotency genes, or addition of growth factors or cytokines or cellular signaling moieties that induce or maintain endogenous pluripotent states. The use of retroviruses to reprogram cells to pluripotent states presents dangers that recall the gene therapy trials for immune deficiency. Excision techniques such as Cre-lox or the piggyBac

WHAT IS CLAIMED IS:

1. A method for producing a therapeutic recombinant biologic polypeptide or protein for treating a disease comprising transfecting a synthetically-produced Pluripotent Stem Cell (spPSC) with a nucleic acid that encodes for said therapeutic recombinant biologic polypeptide or protein, under conditions wherein the polypeptide or protein is expressed by said spPSC, wherein the spPSC is produced from a cell of an animal.

2. The method of claim 1 wherein the therapeutic biologic polypeptide or protein is selected from the group consisting of erythropoietin, factor VIII, factor IX, thrombin, an antibody or antibody fragment, alpha interferon, interferon alpha 2A and 2B, beta interferon, growth hormone, antihemophilic factor, G-CSF, GM-CSF, a soluble receptor, TGF-ß, bone morphogenic proteins (BMP), TGFα, interleukin 2, ß-glucocerebrosidase or an analogue thereof, alpha1-proteinase inhibitor, fibrin, fibrinogen, von Willebrand factor, imiglucerase, agalsidase beta, laronidase, glucosidase alpha, thyrotropin alpha, and thymosin alpha.

3. The method of claim 1 further comprising immortalizing the transfected spPSC.

4. The method of claim 3 further comprising inducing differentiation of the transfected, immortalized spPSCs.

5. The method of claim 1 further comprising inducing differentiation of the transfected spPSCs.

6. The method of claim 5 further comprising immortalizing the transfected, differentiated cells.

7. The method of claim 1 wherein the spPSC is selected from the group consisting of transfected non-Pluripotent Stem Cells, pluripotent stem cells produced by Somatic cell nuclear transfer (SCNT pluripotent stem cells), pluripotent stem cells produced by Altered nuclear transfer, oocyte assisted reprogramming (ANT-OAR pluripotent stem cells) and pluripotent stem cells produced by parthenogenesis (PGA pluripotent).

8. The method of claim 1, wherein the therapeutic recombinant biologic polypeptide or protein is selected from the group consisting of: growth hormones, cytotoxic biologics, and monoclonal antibodies.

9. Use of a therapeutic recombinant biologic polypeptide or protein for the manufacture of a medicament for treating an animal suffering from a disease, wherein the therapeutic recombinant biologic polypeptide or protein is produced by:

(a) identifying an animal suffering from the disease;

(b) providing a synthetically-produced Pluripotent Stem Cell (spPSC) derived from the species of animal suffering from the disease; and

(c) producing the therapeutic recombinant biologic polypeptide or protein by transfecting the spPSC with a nucleic acid that encodes for the therapeutic recombinant biologic polypeptide or protein, under conditions wherein the polypeptide or protein is expressed by said spPSC.

10. The use of claim 9 wherein the therapeutic biologic polypeptide or protein is selected from the group consisting of erythropoietin, factor VIII, factor IX, thrombin, an antibody or antibody fragment, alpha interferon, interferon alpha 2A and 2B, beta interferon, growth hormone, antihemophilic factor, G-CSF, GM-CSF, a soluble receptor, TGF-ß, bone morphogenic proteins (BMP), TGFα, interleukin 2, ß-glucocerebrosidase or an analogue thereof, alpha1-proteinase inhibitor, fibrin, fibrinogen, von Willebrand factor, imiglucerase, agalsidase beta, laronidase, glucosidase alpha, thyrotropin alpha, and thymosin alpha.

11. The use of claim 9 further comprising immortalizing the transfected spPSC.

12. The use of claim 11 further comprising inducing differentiation of the transfected, immortalized spPSCs.

13. The use of claim 9 further comprising inducing differentiation of the transfected spPSC.

14. The use of claim 13 further comprising immortalizing the transfected, differentiated cells.

15. The use of claim 9 wherein the spPSC is selected from the group consisting of: transfected non-Pluripotent Stem Cells, pluripotent stem cells produced by Somatic cell nuclear transfer (SCNT pluripotent stem cells), pluripotent stem cells produced by Altered nuclear

transfer, oocyte assisted reprogramming (ANT-OAR pluripotent stem cells) and pluripotent stem cells produced by parthenogenesis (PGA pluripotent).

16. The use of claim 9, wherein the therapeutic recombinant biologic polypeptide or protein is selected from the group consisting of: growth hormones, cytotoxic biologics, and monoclonal antibodies.

17. The use of claim 9, wherein the disease is selected from the group consisting of diabetes and cancer.

AMENDED CLAIMS
received by the International Bureau on 19 July 2012 (19.07.2012)

WHAT IS CLAIMED IS:

1. A method for producing a recombinant polypeptide or protein comprising transfecting a synthetically-produced Pluripotent Stem Cell (spPSC) with a nucleic acid that encodes for said polypeptide or protein, under conditions wherein the polypeptide or protein is expressed by said stem cell, wherein the spPSC is produced from a cell of an animal.

2. The method of claim 1 wherein the polypeptide or protein is selected from the group consisting of erythropoietin, factor VIII, factor IX, thrombin, an antibody or antibody fragment, alpha interferon, interferon alpha 2A and 2B, beta interferon, growth hormone, antihemophilic factor, G-CSF, GM-CSF, a soluble receptor, TGF-ß, bone morphogenic proteins (BMP), TGFα, interleukin 2, ß-glucocerebrosidase or an analogue thereof, alpha1-proteinase inhibitor, fibrin, fibrinogen, von Willebrand factor, imiglucerase, agalsidase beta, laronidase, glucosidase alpha, thyrotropin alpha, and thymosin alpha.

3. The method of claim 1 wherein the antibody or antibody fragment binds to a target, wherein said target is selected from the group consisting of a tumor necrosis factor (TNF) molecule, a tumor necrosis factor receptor (TNFR), a growth factor receptor, a vascular endothelial growth factor (VEGF) molecule, interleukin 1, interleukin 4, interleukin 6, interleukin 11, interleukin 12, gamma interferon, receptor activator of nuclear factor kappa-B ligand (RANKL) and Blys.

4. The method of claim 1 wherein said soluble receptor binds to a target selected from the group consisting of TNFα, TNFβ and Blys.

5. The method of claim 1 further comprising immortalizing the transfected spPSC.

7. The method of claim 5 further comprising inducing differentiation of the transfected, immortalized spPSCs.

8. The method of claim 1 further comprising inducing differentiation of the transfected spPSCs.

9. The method of claim 7 further comprising immortalizing the transfected, differentiated cells.

10. The method of claim 1 wherein the spPSC is selected from the group consisting of induced Pluripotent Stem Cells, pluripotent stem cells produced by Somatic cell nuclear transfer (SCNT pluripotent stem cells), pluripotent stem cells produced by Altered nuclear transfer, oocyte assisted reprogramming (ANT-OAR pluripotent stem cells) and pluripotent stem cells produced by parthenogenesis (PGA pluripoten).