CELL WASH, ISOLATION AND CRYOPRESERVATION OF FROZEN UMBILICAL CORD BLOOD

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application includes a claim of priority under 35 U.S.C. §119(e) to U.S. provisional patent application No. 63/551,579, filed February 9, 2024, and provisional patent application No. 63/551,582, filed February 9, 2024, the entireties of both are hereby incorporated by reference.

FIELD OF INVENTION

[0002] This invention relates to cell wash, isolation and cryopreservation of frozen umbilical cord blood which can allow for reprogramming iPSCs from cord blood.

BACKGROUND

[0003] All publications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

[0004] Cord blood provide a source for cells that can be utilized for reprogramming into induced pluripotent stem cells (iPSCs) among other things. However, development of successful and reliable processes for cell wash, isolation and cryopreservation of frozen umbilical cord blood to allow their further use, such as reprogramming, and at the same time conform to current good manufacturing practices (cGMP) to manufacture therapeutic cell products are difficult. Thus, there remains a need in the art for these processes.

SUMMARY OF THE INVENTION

[0005] The following embodiments and aspects thereof are described and illustrated in conjunction with compositions and methods which are meant to be exemplary and illustrative, not limiting in scope.

[0006] Various embodiments provide for a method of washing a frozen cord blood sample and isolating cells, comprising

(a) thawing a frozen cord blood unit (CBU), comprising: obtaining CBU; transferring the frozen CBU into a container containing sterile water placed in a 35°C-39°C bath to thaw into cord blood;

(b)(i) performing a first prelysis wash, comprising: transferring the cord blood into a first tube; transferring at least a portion the cord blood into a second tube containing wash buffer ("cells tube"); centrifuging the cells tube at 500xG-1000xG for 8-12 minutes at 4°C-20°C resulting in supernatant and pellet;

(b)(ii) performing a second prelysis wash, comprising removing the supernatant from the centrifuged cells tube; resuspending the pellet with 15-45 mL of wash buffer; centrifuging the cells tube containing the resuspended pellet at 500xG-1000xG for 8-12 minutes at 4°C-20°C resulting in supernatant and pellet;

(b)(iii) optionally, performing a third prelysis wash, comprising: removing the supernatant from the centrifuged cells tube; resuspending the pellet with 30mL of wash buffer; centrifuging the cells tube containing the resuspended pellet at 500xG-1000xG for 8-12 minutes at 4°C-20°C resulting in supernatant and pellet;

(c) performing lysis of red blood cells (RBC), comprising: removing the supernatant from the cells tube; resuspending the pellet with 20-60 mL of lysis buffer; incubating the cells tube for up to 15 minutes at room temperature; centrifuging the cells tube at 500xG-1000xG for 8-12 minutes at 4°C-20°C resulting in supernatant and pellet;

(d) performing a post lysis wash, comprising: removing the supernatant from the cells tube; resuspending the pellet with 15-45 mL of wash buffer; centrifuging the cells tube at 500xG-1000xG for 8-12 minutes at 15-25°C resulting in supernatant and pellet;

(e) straining the cells, comprising: removing the supernatant from the cells tube; adding StemSpan AOF to the cells tube; resuspending the pellet into a cell suspension; optionally, pooling the cell suspension in the cells tube with one or more additional cell suspension into one tube; transferring StemSpan AOF into a third tube ("strain tube") through a 30μm-100μm sterile cell strainer, coating the surface area of the cell strainer; transferring the cell suspension from the cells tube through the cell strainer into the strain tube.

[0007] In various embodiments, the method can comprise

(a) thawing a frozen cord blood unit (CBU), comprising: obtaining CBU; transferring the frozen CBU into a container containing sterile water placed in an about 37°C bath to thaw into cord blood;

(b)(i) performing a first prelysis wash, comprising: transferring the cord blood into a first conical tube; transferring at least a portion the cord blood into a second conical tube containing wash

buffer ("cells tube"); centrifuging the cells tube at about 600xG for 10 minutes at about 4°C resulting in supernatant and pellet;

(b)(ii) performing a second prelysis wash, comprising removing the supernatant from the centrifuged cells tube; resuspending the pellet with about 30mL of wash buffer; centrifuging the cells tube containing the resuspended pellet at about 600xG for about 10 minutes at about 4°C resulting in supernatant and pellet;

(b)(iii) optionally, performing a third prelysis wash, comprising: removing the supernatant from the centrifuged cells tube; resuspending the pellet with about 30mL of wash buffer; centrifuging the cells tube containing the resuspended pellet at about 600xG for about 10 minutes at about 4°C resulting in supernatant and pellet;

(c) performing lysis of red blood cells (RBCs), comprising: removing the supernatant from the cells tube; resuspending the pellet with about 40mL of lysis buffer; incubating the cells tube for about 10-15 minutes room temperature; centrifuging the cells tube at about 600xG for about 10 minutes at about 4°C resulting in supernatant and pellet;

(d) performing a post lysis wash, comprising: removing the supernatant from the cells tube; resuspending the pellet with about 30 mL of wash buffer; centrifuging the cells tube at about 600xG for about 10 minutes at about room temperature resulting in supernatant and pellet;

(e) straining the cells, comprising: removing the supernatant from the cells tube; adding about 5mL of StemSpan AOF to the cells tube; resuspending the pellet into a cell suspension; optionally, pooling the cell suspension in the cells tube with one or more additional cell suspension into one tube; transferring about 1mL of StemSpan AOF into a third conical tube ("strain tube") through an about 40 μm sterile cell strainer, coating the surface area of the cell strainer; transferring the cell suspension from the cells tube through the cell strainer into the strain tube.

[0008] In various embodiments, the first tube, second tube, and/or third tube can be a conical tube.

[0009] In various embodiments, the method can further comprise transferring, under aseptic conditions, the thawed CBU into a biological safety cabinet (BSC).

[0010] In various embodiments, pooling the cell suspension in the cells tube ("first cells tube") with one or more additional cell suspension into one tubes, can comprise: adding about 5 ml of StemSpan AOF to the first cells tube and to one or more additional cells tube; resuspending the pellet in the first cells tube and resuspending the pellet in the one or more additional cells tubes; transferring the cell suspension from the one or more additional cells tubes to the first cells tube; adding about 7.5 mL of StemSpan AOF into the one or more additional cells tubes to wash and collect residual cells, and

transferring the suspension from the one or more additional cells tubes to the first cells tube; and optionally, resuspending any remaining aggregates or clumps.

[0011] In various embodiments, the method can further comprise adding cryopreservation solution to the strain tube for cryopreservation. In various embodiments, the method can further comprise cryopreserving the cells.

[0012] In various embodiments, the wash buffer can comprise DPBS:HSA:EDTA ratio of 366:7.5:1.5, the concentrations of HSA and EDTA are 0.5% HSA and 2 mM EDTA. In various embodiments, the concentration of the lysis buffer can be about 4000U/mL of DNase (4KU). In various embodiments, the lysis buffer can be filtered and can result in a concentration of about 1U/mL.

[0013] Various embodiments provide a cell suspension comprising: isolated cells from cord blood sample in accordance with the embodiments of the present invention; and StemSpan AOF.

[0014] In various embodiments, the isolated cells from cord blood sample is isolated by any one of the methods of the present invention..

[0015] Various embodiments provide for a method of automated filling and cryopreserving isolated cells from cord blood sample , comprising: (a) obtaining isolated cells from cord blood sample, wherein the isolated cells from cord blood sample have been spun down in a tube; (b) removing supernatant from pellet; (c) resuspending the pellet; (d) adding CS10 to the tube and resuspending the pellet into an isolated cells from cord blood sample suspension; (e) optionally, combing the isolated cells from cord blood sample suspension with one or more isolated cells from cord blood sample suspensions; (f) adding additional CS10 to bring the volume of the isolated cells from cord blood sample suspension to 15-35 mL; (g) transferring the isolated cells from cord blood sample suspension to a 125-375 mL sterile bottle; (h) adding additional CS10 to bring the volume of the isolated cells from cord blood sample suspension to 100-200 mL; (i) inserting a tube into the bottle comprising the isolated cells from cord blood sample suspension and priming the tubing until some isolated cells from cord blood sample suspension is dispensed into a reservoir; (j) placing a multi-vial rack on a rack nest; and (k) selecting a desired dispensing volume on an automated filling machine; (l) operating the automated filling machine to fill the vials in the multi-vial rack; and (m) transferring the filled cryovial to the controlled rate freezer (CRF).

[0016] In various embodiments, the automated filling and cryopreserving isolated cells from cord blood sample, can comprise: (a) obtaining harvested isolated cells from cord blood sample, wherein the isolated cells from cord blood sample have been spun down in a tube; (b) removing supernatant from pellet using a serological pipette; (c) resuspending the pellet by flicking the tube; (d) adding about 1mL of CS10 to the tube and pipette up and down no more than 3 times to break up the pellet resulting in an isolated cells from cord blood sample suspension ; (e) optionally, combing the

isolated cells from cord blood sample suspension with one or more iPSC suspensions; (f) adding additional CS10 to bring the volume of the isolated cells from cord blood sample suspension to about 25 mL; (g) transferring the isolated cells from cord blood sample suspension to a 250 mL sterile bottle; (h) adding additional CS10 to bring the volume of the isolated cells from cord blood sample suspension to about 157 mL; (i) inserting a tube into the bottle comprising the isolated cells from cord blood sample suspension and priming the tubing until some isolated cells from cord blood sample suspension is dispensed into a reservoir; (j) placing a 48-vial rack on a rack nest; and (k) selecting a desired dispensing volume of 1000µL on the automated filling machine; (l) operating the automated filling machine to fill the vials in the multi-vial rack; and (m) transferring the filled cryovial to the controlled rate freezer (CRF).

[0017] In various embodiments, the method can further comprise allowing a sample probe to reach about 4°C; once at about 4°C, running the CRF; upon completion of CRF, transferring the cryovials to an LN2 tank.

[0018] In various embodiments, transferring the cryovial to an LN2 tank, can comprise first transferring the vials to a container with dry ice and then transferring the vial to an LN2 tank.

[0019] Various embodiments provide for a cell suspension comprising: isolated cells from cord blood sample; and CS10 medium.

[0020] In various embodiments, the isolated cells from cord blood sample can be isolated by any one of the methods of the present invention and cryopreserved by any one of the methods of the present invention.

[0021] Various embodiments provide for a method of processing and enrichment of cord blood unit (CBU) isolated cell fraction, comprising:

(a) cell fraction thawing and seeding, comprising: adding StemSpan AOF into a vial containing frozen CBU isolated cell fraction; thawing each vial; transferring the thawed vial contents into a tube ("cells tube"); using Stem Span AOF to rinse the vial and collect residual cells; transferring the suspension into the cells tube; adding StemSpan AOF into the cells tube; centrifuge the cells tube at 500xG-1000xG for 5-15 minutes resulting in supernatant and pellet; discarding the supernatant; resuspending the pellet with complete media; optionally pooling the cell suspension in the cells tube ("first cells tube") with one or more additional cell suspension into one tube;

(b) cell fraction feeding 1, comprising: transferring a calculated volume of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into a flask; transferring a calculated volume of the cell suspension to the flask; incubating the cell suspension in the flask;

(c) cell fraction feeding 2, comprising: collecting the cell suspension in the flask and dispense it down the bottom of the flask 2-3 times; transferring the suspension from the flask into a

conical tube; adding complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into the flask to rinse and collect residual cells; transferring the suspension from the flask into the conical tube; centrifuging the conical tube at 500-1000xG for 10 minutes at room temperature resulting in supernatant and pellet; remove the supernatant, leaving behind approximately 5 mL; resuspending the pellet with 30 mL of Stem Span Complete Media; transferring the suspension into a flask; incubating the cell suspension in the flask;

(d) cell fraction feeding 3, comprising: collecting the cell suspension in the flask and dispense it down the bottom of the flask 2-3 times; transferring the suspension from the flask into a conical tube; adding 5 mL of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into the flask to rinse and collect residual cells; transferring the suspension from the flask into the conical tube; centrifuging the conical tube at 500xG-1000xG for 5-15 minutes resulting in supernatant and pellet; remove the supernatant, leaving behind approximately 5 mL; resuspending the pellet with Stem Span Complete Media; transferring the suspension into a flask; incubating the cell suspension in the flask.

[0022] In various embodiments, processing and enrichment of cord blood unit (CBU) isolated cell fraction, can comprise:

(a) cell fraction thawing and seeding, comprising: adding about 4.5 mL of StemSpan AOF into a vial containing frozen CBU isolated cell fraction; thawing each vial in an about 37°C beaker in a bead bath; transferring the thawed vial contents into a tube ("cells tube"); using about 4.5 mL of Stem Span AOF to rinse the vial and collect residual cells; transferring the suspension into the cells tube; adding about 31.5 mL of StemSpan AOF into the cells tube to yield a total volume of about 45mL; centrifuge the cells tube at about 600xG for about 10 minutes at about room temperature resulting in supernatant and pellet; discarding the supernatant; resuspending the pellet with about 1 mL of complete media; optionally pooling the cell suspension in the cells tube ("first cells tube") with one or more additional cell suspension into one tube;

(b) cell fraction feeding 1, comprising: transferring a calculated volume of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into a flask; transferring a calculated volume of the cell suspension to the flask; incubating the cell suspension in the flask;

(c) cell fraction feeding 2, comprising: collecting the cell suspension in the flask and dispense it down the bottom of the flask about 2-3 times; transferring the suspension from the flask into a conical tube; adding about 5 mL of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into the flask to rinse and collect residual cells; transferring the suspension from the flask into the conical tube; centrifuging the conical tube at about 600xG for about 10 minutes at room temperature resulting in supernatant and pellet; remove the supernatant, leaving

behind approximately about 5 mL; resuspending the pellet with about 30 mL of complete media; transferring the suspension into a flask; incubating the cell suspension in the flask;

(d) cell fraction feeding 3, comprising: collecting the cell suspension in the flask and dispense it down the bottom of the flask about 2-3 times; transferring the suspension from the flask into a conical tube; adding about 5 mL of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into the flask to rinse and collect residual cells; transferring the suspension from the flask into the conical tube; centrifuging the conical tube at about 600xG for about 10 minutes at room temperature resulting in supernatant and pellet; remove the supernatant, leaving behind approximately about 5 mL; resuspending the pellet with about 30 mL of complete media; transferring the suspension into a flask; incubating the cell suspension in the flask.

[0023] In various embodiments, the method can further comprise cell fraction harvesting on day of reprogramming.

[0024] In various embodiments, the complete media comprising StemSpan AOF and StemSpan CD34+ Expansion Supplement can be an about 9:1 ratio of StemSpan AOF:StemSpan CD34+ Expansion Supplement.

[0025] In various embodiments, optionally pooling the cell suspension in the cells tube ("first cells tube") with one or more additional cell suspension into one tube, comprises transferring the cell suspension from the one or more additional cells tubes to the first cells tube; adding about 1 mL of complete media into the one or more additional cells tubes to wash and collect residual cells, and transferring the suspension from the one or more additional cells tubes to the first cells tube.

[0026] Various embodiments provide for a method of washing a frozen cord blood sample, isolating cells, and processing and enrichment of cord blood unit (CBU) isolated cell fraction, comprising performing the method steps as described herein.

[0027] Various embodiments provide for a quantity of hematopoietic stem/progenitor cells isolated from cord blood, wherein the hematopoietic stem/progenitor cells comprise cells selected from erythroid progenitors, monocyte and macrophages, granulocyte progenitors and lymphoid cells or combinations thereof, and wherein the hematopoietic stem/progenitor cells express one or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22.

[0028] In various embodiments, the hematopoietic stem/progenitor cells express one or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22.

[0029] Various embodiments provide for a cell suspension comprising: isolated cell fraction (ICF) from Cord Blood Units (CBUs); and complete media, wherein complete media comprises StemSpan AOF and StemSpan CD34+ Expansion Supplement.

[0030] In various embodiments, the ICF comprises hematopoietic stem/progenitor cells selected from erythroid progenitors, monocyte and macrophages, granulocyte progenitors and lymphoid cells or combinations thereof, and wherein the hematopoietic stem/progenitor cells express one or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22.

[0031] Other features and advantages of the invention will become apparent from the following detailed description, taken in conjunction with the accompanying drawings, which illustrate, by way of example, various features of embodiments of the invention.

BRIEF DESCRIPTION OF THE FIGURES

[0032] Exemplary embodiments are illustrated in referenced figures. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive.

[0033] Figure 1 shows an example of clear separation between the pellet containing red blood cells (RBCs) and the supernatant.

[0034] Figure 2 shows an example of grouping the vials to thaw. In this example, 16 total vials have been split into 4 distinct thawing groups, each with 4 vials.

[0035] Figure 3 shows an example illustrating the labeling format for 50mL “Cells” tubes. A maximum of four (4) 50 mL conical tubes may be in a group.

[0036] Figure 4 shows an example depicting that one vial from the group will be transferred into one 50 mL conical tube in the respective group.

[0037] Figure 5 shows an example outlining the method for pooling the cells into one tube. In this case, the suspension from “Cells 1.2”, “Cells 1.3”, and “Cells 1.4” will all be transferred into the “Cells 1.1” tube.

[0038] Figure 6 shows an example displaying the pooling the rinse suspension into one tube. In this case, the suspension from “Cells 1.2”, “Cells 1.3”, and “Cells 1.4” will all be transferred into the “Cells 1.1” tube.

[0039] Figure 7 shows a representative image detailing how to pool the suspension from all vial thaw groups into one tube. In this example, the suspension from the “Cells 2.1”, “Cells 3.1”, and “Cells 4.1” tubes will all be transferred into the “Cells 1.1” tube.

[0040] Figure 8A-8B show Uniform Manifold Approximation and Projection (UMAP) clustering of single cell data that separates the distinct sample sets as shown in the legend.

[0041] Figure 9 shows distinct subclusters and separation across all samples.

[0042] Figure 10 shows dot plot of specific marker genes across a matrix of clusters as defined in Figure 9 and categorizing them as specific cell type.

[0043] Figure 11 shows the CBU Post Wash Violin Plot and the CBU Post Enrichment Violin Plot.

[0044] Figures 12-16 show known blood and hematopoietic stem/ progenitor marker genes expressed by each of the sample sets.

DESCRIPTION OF THE INVENTION

[0045] All references cited herein are incorporated by reference in their entirety as though fully set forth. Unless defined otherwise, technical, and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following terms are defined below.

[0046] As used herein the term "about" when used in connection with a referenced numeric indication means the referenced numeric indication plus or minus up to 5% of that referenced numeric indication, unless otherwise specifically provided for herein. In various embodiments, the term "about" when used in connection with a referenced numeric indication can mean the referenced numeric indication plus or minus up to 4%, 3%, 2%, 1%, 0.5%, or 0.25% of that referenced numeric indication, *if* specifically provided for in the claims.

[0047] "Room temperature" as used herein refers to a temperature between 15°C to 25°C. In various embodiments, "room temperature" refers to a temperature between 18°C to 22°C, or between 19°C to 21°C, or about 20°C, *if* specifically provided for in the claims.

[0048] StemSpan AOF referenced herein is an animal origin free media. It is a cGMP medium, for culture and expansion of human hematopoietic cells. It contains only recombinant proteins and synthetic components, and does not contain serum or other human- or animal-derived components.

[0049] StemSpan CD34+ expansion supplement is a serum-free culture supplement for expansion of human CD34+ hematopoietic cells. It contains a combination of recombinant human cytokines and other additives formulated to selectively promote the expansion of CD34+ cells isolated from human cord blood (CB) or bone marrow (BM) samples.

Acronym/Term	Definition
BPR	Batch Production Record
BSC	Biosafety Cabinet
CBC	Cedars-Sinai Biomanufacturing Center
CBU	Cord Blood Unit
cGMP(s)	Current Good Manufacturing Practice(s)
CoA	Certificate of Analysis
CRF	Controlled Rate Freezer
DPBS -/-	Dulbecco's Phosphate-Buffered Saline without Calcium and without Magnesium
EDTA	Ethylenediaminetetraacetic Acid
EM	Environmental Monitoring
HSA	Human Serum Albumin
ICF	Isolated Cell Fraction
ID	Identification
IPA	Isopropyl Alcohol
LN2	Liquid nitrogen
MFG	Manufacturing
MPR	Master Production Record
PeM	Personnel Monitoring
PD	Process Development
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
SOP	Standard Operating Procedure

[0050] The procedures described herein for the washing a frozen cord blood sample and isolating cells, processing and cryopreservation of viable cell fraction from cryopreserved/frozen cord blood in preparation for downstream cell manufacturing activities including reprogramming to iPSCs at CBC following current Good Manufacturing Practice (cGMP).

[0051] Various embodiments of the present invention provide for a method of washing a frozen cord blood sample and isolating cells. These methods remove RBCs and other components that are part of frozen cord blood, isolate and enrich a unique composition of viable hematopoietic stem/progenitor cells from cord blood, including erythroid progenitors, monocyte and macrophages, granulocyte progenitors and lymphoid cells capable of being transformed into iPSCs. The method comprises:

(a) thawing a frozen cord blood unit (CBU), comprising:

obtaining CBU;

transferring the frozen CBU into a container containing sterile water placed in a 35°C-39°C bath to thaw into cord blood;

(b)(i) performing a first prelysis wash, comprising:

transferring the cord blood into a first tube;

transferring at least a portion the cord blood into a second tube containing wash buffer ("cells tube");

centrifuging the cells tube at 500xG-1000xG for 8-12 minutes at 4°C-20°C resulting in supernatant and pellet;

(b)(ii) performing a second prelysis wash, comprising

removing the supernatant from the centrifuged cells tube;

resuspending the pellet with 15-45 mL of wash buffer;

centrifuging the cells tube containing the resuspended pellet at 500xG-1000xG for 8-12 minutes at 4°C-20°C resulting in supernatant and pellet;

(b)(iii) optionally, performing a third prelysis wash, comprising:

removing the supernatant from the centrifuged cells tube;

resuspending the pellet with 15-45 of wash buffer;

centrifuging the cells tube containing the resuspended pellet at 500xG-1000xG for 8-12 minutes at 4°C-20°C resulting in supernatant and pellet;

(c) performing lysis of red blood cells (RBCs), comprising:

removing the supernatant from the cells tube;

resuspending the pellet with 20-60 mL of lysis buffer;

incubating the cells tube for up to 15 minutes at 15-25°C;

centrifuging the cells tube at 500xG-1000xG for 8-12 minutes at 4°C-20°C resulting in supernatant and pellet;

(d) performing a post lysis wash, comprising:

removing the supernatant from the cells tube;

resuspending the pellet with 15-45 mL of wash buffer;

centrifuging the cells tube at 500xG-1000xG for 8-12 minutes at 15°C-25°C resulting in supernatant and pellet; and

(e) straining the cells, comprising:

removing the supernatant from the cells tube;

adding StemSpan AOF media to the cells tube;

resuspending the pellet into a cell suspension;

optionally, pooling the cell suspension in the cells tube with one or more additional cell suspension into one tube;

transferring StemSpan AOF into a third tube ("strain tube") through a 30µm-100µm sterile cell strainer, coating the surface area of the cell strainer;

transferring the cell suspension from the cells tube through the cell strainer into the strain tube.

[0052] In various embodiments, in step (a) the bath is at 36°C-38°C bath to thaw into cord blood.

[0053] In various embodiments, in step (b)(i) the cells tube is centrifuged at 550xG-650xG for 9-11 minutes at 4°C-10°C resulting in supernatant and pellet. In various embodiments, in step (b)(ii) the cells tube is centrifuged at 550xG-650xG for 9-11 minutes at 4°C-10°C resulting in supernatant and pellet. In various embodiments, in step (b)(iii) the cells tube is centrifuged at 550xG-650xG for 9-11 minutes at 4°C-10°C resulting in supernatant and pellet.

[0054] In various embodiments, in step (c) the pellet is resuspended with 30-50 mL of lysis buffer. In various embodiments, in step (c) the cells tube is incubated for 12-15 minutes at 18-22°C. In various embodiments, in step (c) the cells tube is centrifuged at 550xG-650xG for 9-11 minutes at 4°C-10°C resulting in supernatant and pellet.

[0055] In various embodiments, in step (d) the pellet is resuspended with 20-40 mL of wash buffer. In various embodiments, in step (d) the cells tube is centrifuged at 550xG-650xG for 9-11 minutes at 18°C-22°C resulting in supernatant and pellet.

[0056] In various embodiments, in step (e) 3-7mL of StemSpan AOF is added to the cells tube.

[0057] In various embodiments, in step (e) about 1mL of StemSpan AOF is transferred into a third tube ("strain tube") through a 30µm-50µm sterile cell strainer, coating the surface area of the cell strainer.

[0058] In various embodiments, the method comprises

(a) thawing a frozen cord blood unit (CBU), comprising:

obtaining CBU;

transferring the frozen CBU into a container containing sterile water placed in an about 37°C bath to thaw into cord blood;

(b)(i) performing a first prelysis wash, comprising:

transferring the cord blood into a first conical tube;

transferring at least a portion the cord blood into a second conical tube containing wash buffer ("cells tube");

centrifuging the cells tube at about 600xG for about 10 minutes at about 4°C resulting in supernatant and pellet;

(b)(ii) performing a second prelysis wash, comprising

removing the supernatant from the centrifuged cells tube;

resuspending the pellet with about 30mL of wash buffer;

centrifuging the cells tube containing the resuspended pellet at about 600xG for about 10 minutes at about 4°C resulting in supernatant and pellet;

(b)(iii) optionally, performing a third prelysis wash, comprising:

removing the supernatant from the centrifuged cells tube;

resuspending the pellet with about 30mL of wash buffer;

centrifuging the cells tube containing the resuspended pellet at about 600xG for about 10 minutes at about 4°C resulting in supernatant and pellet;

(c) performing lysis of red blood cells (RBCs), comprising:

removing the supernatant from the cells tube;

resuspending the pellet with about 40mL of lysis buffer;

incubating the cells tube for about 10-15 minutes room temperature;

centrifuging the cells tube at about 600xG for about 10 minutes at about 4°C resulting in supernatant and pellet;

(d) performing a post lysis wash, comprising:

removing the supernatant from the cells tube;

resuspending the pellet with about 30 mL of wash buffer;

centrifuging the cells tube at about 600xG for about 10 minutes at room temperature resulting in supernatant and pellet;

(e) straining the cells, comprising:

removing the supernatant from the cells tube;

adding about 5mL of StemSpan AOF to the cells tube;

resuspending the pellet into a cell suspension;

optionally, pooling the cell suspension in the cells tube with one or more additional cell suspension into one tube;

transferring about 1mL of StemSpan AOF into a third conical tube ("strain tube") through a about 40 μm sterile cell strainer, coating the surface area of the cell strainer; and

transferring the cell suspension from the cells tube through the cell strainer into the strain tube.

[0059] In various embodiments, the method further comprises transferring, under aseptic conditions, the thawed CBU into a biological safety cabinet (BSC).

[0060] In various embodiments, the CBU is cord blood units specially formulated for human therapeutic purposes. In various embodiments, the CBU is an FDA approved. In various embodiments, the CBU is approved by regulatory agencies similar to the FDA, for example, European Medicines Agency (EMA), Medicines and Healthcare Products Regulatory Agency (MHRA), National Medical Products Administration (NMPA). Exemplary CBU include but is not limited to DUCORD (HPC Cord Blood) (see e.g., www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/ducord-hpc-cord-blood, last accessed February 4, 2025.) Additional examples include but are not limited to ALLOCORD (HPC Cord Blood), CLEVECORD (HPC Cord Blood), HEMACORD (HPC, cord blood), HPC, Cord Blood (Clinimmune Labs, University of Colorado Cord Blood Bank), HPC, Cord Blood - MD Anderson Cord Blood Bank, HPC, Cord Blood – LifeSouth, HPC, Cord Blood – Bloodworks, and HPC, Cord Blood (REGENECYTE), (see e.g., www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products, last accessed February 4, 2025).

[0061] In various embodiments, the method further comprises adding cryopreservation solution to the strain tube for cryopreservation. In various embodiments, the method further comprises cryopreserving the cells.

[0062] In various embodiments, the first tube, second tube, and/or third tube is a conical tube. In various embodiments, the first tube, second tube, and/or third tube is a sterile plasticware capable of accommodating the volume and capable of being centrifuged.

[0063] In various embodiments, pooling the cell suspension in the cells tube ("first cells tube") with one or more additional cell suspension into one tubes, comprises: adding StemSpan AOF to the first cells tube and to one or more additional cells tube; resuspending the pellet in the first cells tube and resuspending the pellet in the one or more additional cells tubes; transferring the cell suspension from the one or more additional cells tubes to the first cells tube; adding StemSpan AOF into the one or more additional cells tubes to wash and collect residual cells, and transferring the suspension from the one or more additional cells tubes to the first cells tube; and optionally, resuspending any remaining aggregates or clumps.

[0064] In various embodiments, pooling the cell suspension in the cells tube ("first cells tube") with one or more additional cell suspension into one tubes, comprises adding 2-8 ml of StemSpan AOF to the first cells tube and to one or more additional cells tube; resuspending the pellet in the first cells tube and resuspending the pellet in the one or more additional cells tubes; transferring the cell suspension from the one or more additional cells tubes to the first cells tube; adding 5-10 mL of StemSpan AOF into the one or more additional cells tubes to wash and collect residual cells, and transferring the suspension from the one or more additional cells tubes to the first cells tube; and optionally, resuspending any remaining aggregates or clumps.

[0065] In various embodiments, pooling the cell suspension in the cells tube ("first cells tube") with one or more additional cell suspension into one tubes, comprises adding about 5 ml of StemSpan AOF to the first cells tube and to one or more additional cells tube; resuspending the pellet in the first cells tube and resuspending the pellet in the one or more additional cells tubes; transferring the cell suspension from the one or more additional cells tubes to the first cells tube; adding about 7.5 mL of StemSpan AOF into the one or more additional cells tubes to wash and collect residual cells, and transferring the suspension from the one or more additional cells tubes to the first cells tube; and optionally, resuspending any remaining aggregates or clumps.

[0066] In various embodiments, the wash buffer comprises DPBS:HSA:EDTA ratio of 275-458 : 5-10 : 1.1-1.9, the concentrations of HSA and EDTA are 0.375-0.625% HSA and 1.5-2.5 mM EDTA.

[0067] In various embodiments, the wash buffer comprises DPBS:HSA:EDTA ratio of 329-403 : 6.75-8.25 : 1.35-1.65, the concentrations of HSA and EDTA are 0.45-0.55% HSA and 1.8-2.2 mM EDTA.

[0068] In various embodiments, the wash buffer comprises DPBS:HSA:EDTA ratio of about 366 : 7.5 : 1.5, the concentrations of HSA and EDTA are about 0.5% HSA and about 2 mM EDTA.

[0069] In various embodiments, the concentration of the lysis buffer is 3500-4500U/mL of DNase (4KU). In various embodiments, the lysis buffer is filtered and results in a concentration of 0.5-1.5U/mL.

[0070] In various embodiments, the concentration of the lysis buffer is about 4000U/mL of DNase (4KU). In various embodiments, the lysis buffer is filtered and results in a concentration of about 1U/mL.

[0071] In various embodiments, the isolated, or isolated and enriched cells from cord blood wash sample express one or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22. In various embodiments, the isolated and enriched cells from cord blood sample

express 5 or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22. In various embodiments, the isolated cells from cord blood sample express 7 or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22. In various embodiments, the isolated cells from cord blood sample express hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22.

[0072] In various embodiments, these isolated cells are the post wash CBU Wash sample. In various embodiments, these isolated cells are the isolated and enriched CBU cells after culturing them for at least 4 days.

[0073] Various embodiments provide for a cell suspension comprising: isolated cells from cord blood sample; and StemSpan AOF. In various embodiments, the isolated cells from cord blood sample are isolated by any one of the methods of the present invention described herein.

[0074] In various embodiments, the isolated and enriched cells from cord blood sample express one or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22. In various embodiments, the isolated cells from cord blood sample express 5 or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22. In various embodiments, the isolated cells from cord blood sample express 7 or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22. In various embodiments, the isolated cells from cord blood sample express hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22.

[0075] In various embodiments, these isolated cells are the post wash CBU Wash sample. In various embodiments, these isolated cells are the isolated and enriched CBU cells after culturing them for 4 days.

[0076] Various embodiments provide for a method of automated filling and cryopreserving isolated cells from cord blood sample, comprising:

(a) obtaining isolated cells from cord blood sample, wherein the isolated cells from cord blood sample have been spun down in a tube;

(b) removing supernatant from pellet;

(c) resuspending the pellet;

(d) adding CS10 to the tube and resuspending the pellet into an isolated cells from cord blood sample suspension;

(e) optionally, combing the isolated cells from cord blood sample suspension with one or more isolated cells from cord blood sample suspensions;

(f) adding additional CS10 to bring the volume of the isolated cells from cord blood sample suspension to 15-35 mL;

(g) transferring the isolated cells from cord blood sample suspension to a 125-375 mL sterile bottle;

(h) adding additional CS10 to bring the volume of the isolated cells from cord blood sample suspension to 100-200 mL;

(i) inserting a tube into the bottle comprising the isolated cells from cord blood sample suspension and priming the tubing until some isolated cells from cord blood sample suspension is dispensed into a reservoir;

(j) placing a multi-vial rack on a rack nest; and

(k) selecting a desired dispensing volume on an automated filling machine;

(l) operating the automated filling machine to fill the vials in the multi-vial rack; and

(m) transferring the filled cryovial to the controlled rate freezer (CRF).

[0077] In various embodiments, the automated filling and cryopreserving isolated cells from cord blood sample, comprises:

(a) obtaining isolated cells from cord blood sample, wherein the isolated cells from cord blood sample have been spun down in a tube;

(b) removing supernatant from pellet;

(c) resuspending the pellet;

(d) adding 0.5-1.5mL CS10 to the tube and pipette up and down no more than 3 times to break up the pellet resulting in into an isolated cells from cord blood sample suspension;

(e) optionally, combing the isolated cells from cord blood sample suspension with one or more isolated cells from cord blood sample suspensions;

(f) adding additional CS10 to bring the volume of the isolated cells from cord blood sample suspension to 20-30 mL;

(g) transferring the isolated cells from cord blood sample suspension to a 200-300mL sterile bottle;

(h) adding additional CS10 to bring the volume of the isolated cells from cord blood sample suspension to 125-175 mL;

(i) inserting a tube into the bottle comprising the isolated cells from cord blood sample suspension and priming the tubing until some isolated cells from cord blood sample suspension is dispensed into a reservoir;

(j) placing a 24-vial to 72-vial rack on a rack nest; and

(k) selecting a desired dispensing volume on an automated filling machine;

(l) operating the automated filling machine to fill the vials in the multi-vial rack; and

(m) transferring the filled cryovial to the controlled rate freezer (CRF).

[0078] In various embodiments, the automated filling and cryopreserving isolated cells from cord blood sample, comprises:

(a) obtaining harvested isolated cells from cord blood sample, wherein the isolated cells from cord blood sample have been spun down in a tube;

(b) removing supernatant from pellet using a serological pipette;

(c) resuspending the pellet by flicking the tube;

(d) adding about 1mL of CS10 to the tube and pipette up and down no more than 3 times to break up the pellet resulting in an isolated cells from cord blood sample suspension ;

(e) optionally, combing the isolated cells from cord blood sample suspension with one or more iPSC suspensions;

(f) adding additional CS10 to bring the volume of the isolated cells from cord blood sample suspension to about 25 mL;

(g) transferring the isolated cells from cord blood sample suspension to an about 250 mL sterile bottle;

(h) adding additional CS10 to bring the volume of the isolated cells from cord blood sample suspension to about 157 mL;

(i) inserting a tube into the bottle comprising the isolated cells from cord blood sample suspension and priming the tubing until some isolated cells from cord blood sample suspension is dispensed into a reservoir;

(j) placing a 48-vial rack on a rack nest; and

(k) selecting a desired dispensing volume of about 1000μL on the automated filling machine;

(l) operating the automated filling machine to fill the vials in the multi-vial rack; and

(m) transferring the filled cryovial to the controlled rate freezer (CRF).

[0079] In various embodiments, the method further comprises: allowing a sample probe to reach about 4°C; once at about 4°C, running the CRF; upon completion of CRF, transferring the cryovials to an LN2 tank.

[0080] In various embodiments, transferring the cryovial to an LN2 tank, comprises first transferring the vials to a container with dry ice and then transferring the vial to an LN2 tank.

[0081] Various embodiments provide for a cell suspension comprising: isolated cells from cord blood sample; and CS10 medium.

[0082] In various embodiments, the isolated cells from cord blood sample is isolated by any one of the methods of the present invention as described herein and cryopreserved by any one of the methods of the present invention as described herein.

[0083] In various embodiments, the isolated cells from cord blood sample express one or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22. In various embodiments, the isolated cells from cord blood sample express 5 or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22. In various embodiments, the isolated cells from cord blood sample express 7 or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22. In various embodiments, the isolated cells from cord blood sample express hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22.

[0084] In various embodiments, these isolated cells are the post wash CBU Wash sample. In various embodiments, these isolated cells are the isolated and enriched CBU cells after culturing them for 4 days.

[0085] Described herein are also procedures for the processing and enrichment of cells to isolate a viable cell fraction using StemSpan CD34+ Complete Media isolated from frozen Cord Blood Units (CBUs).

[0086] Various embodiments provide for a method of processing and enrichment of cord blood unit (CBU) isolated cell fraction, comprising:

(a) cell fraction thawing and seeding, comprising:

adding StemSpan AOF into a vial containing frozen CBU isolated cell fraction;

thawing each vial;

transferring the thawed vial contents into a tube (“cells tube”);

using Stem Span AOF to rinse the vial and collect residual cells;

transferring the suspension into the cells tube;

adding StemSpan AOF into the cells tube;

centrifuge the cells tube at 500xG-1000xG for 5-15 minutes resulting in supernatant and pellet;

discarding the supernatant;

resuspending the pellet with complete media;

optionally pooling the cell suspension in the cells tube ("first cells tube") with one or more additional cell suspension into one tube;

(b) cell fraction feeding 1, comprising:

transferring a calculated volume of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into a flask;

transferring a calculated volume of the cell suspension to the flask;

incubating the cell suspension in the flask;

(c) cell fraction feeding 2, comprising:

collecting the cell suspension in the flask and dispense it down the bottom of the flask 2-3 times;

transferring the suspension from the flask into a conical tube;

adding complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into the flask to rinse and collect residual cells;

transferring the suspension from the flask into the conical tube;

centrifuging the conical tube at 500-1000xG for 8-12 minutes at room temperature resulting in supernatant and pellet;

removing the supernatant, leaving behind approximately 5 mL;

resuspending the pellet with 30 mL of Stem Span Complete Media;

transferring the suspension into a flask;

incubating the cell suspension in the flask;

(d) cell fraction feeding 3, comprising:

collecting the cell suspension in the flask and dispense it down the bottom of the flask 2-3 times;

transferring the suspension from the flask into a conical tube;

adding 5 mL of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into the flask to rinse and collect residual cells;

transferring the suspension from the flask into the conical tube;

centrifuging the conical tube at 500xG-1000xG for 5-15 minutes resulting in supernatant and pellet;

removing the supernatant, leaving behind approximately 5 mL;

resuspending the pellet with Stem Span Complete Media;

transferring the suspension into a flask;

incubating the cell suspension in the flask.

[0087] In various embodiments in step (a) adding StemSpan AOF into a vial containing frozen CBU isolated cell fraction comprises adding 2.5-6.5 mL of StemSpan AOF. In various embodiments in step (a) thawing each vial comprises thawing each vial in a 34°C-40°C beaker bead bath. In various embodiments in step (a) 2.5-6.5 mL of Stem Span AOF is used to rinse the vial and collect residual cells. In various embodiments in step (a) 26.5-36.5 mL of StemSpan AOF is added into the cells tube. In various embodiments in step (a) the cells tube is centrifuged 550-650xG for 9-11 minutes at room temperature resulting in supernatant and pellet; In various embodiments in step (a) the pellet is resuspended with 0.5-1.5 mL of complete media.

[0088] In various embodiments in step (c) 3-7 mL of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement is added into the flask to rinse and collect residual cells. In various embodiments in step (c) the conical tube is centrifuged at 550-650xG for 9-11 minutes at 18°C-22°C resulting in supernatant and pellet. In various embodiments in step (c) approximately 4-6 mL of supernatant is left behind. In various embodiments in step (c) the pellet is resuspended with 20-40 mL of complete media.

[0089] In various embodiments in step (d) 4-6 mL of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement is added into the flask to rinse and collect residual cells. In various embodiments in step (d) the conical tube is centrifuged at 550-650xG for 9-11 minutes at 18°C-22°C resulting in supernatant and pellet. In various embodiments in step (d) approximately 4-6 mL of supernatant is left behind. In various embodiments in step (d) the pellet is resuspended with 20-40 mL of complete media.

[0090] In various embodiments, processing and enrichment of cord blood unit (CBU) isolated cell fraction, comprises:

(a) cell fraction thawing and seeding, comprising:

adding about 4.5 mL of StemSpan AOF into a vial containing frozen CBU isolated cell fraction;

thawing each vial in an about 37°C beaker in a bead bath;

transferring the thawed vial contents into a tube (“cells tube”);

using about 4.5 mL of Stem Span AOF to rinse the vial and collect residual cells;

transferring the suspension into the cells tube;

adding about 31.5 mL of StemSpan AOF into the cells tube to yield a total volume of 45mL;

centrifuge the cells tube at about 600xG for about 10 minutes at room temperature resulting in supernatant and pellet;

discarding the supernatant;

resuspending the pellet with about 1 mL of complete media;

optionally pooling the cell suspension in the cells tube ("first cells tube") with one or more additional cell suspension into one tube;

(b) cell fraction feeding 1, comprising:

transferring a calculated volume of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into a flask;

transferring a calculated volume of the cell suspension to the flask;

incubating the cell suspension in the flask;

(c) cell fraction feeding 2, comprising:

collecting the cell suspension in the flask and dispense it down the bottom of the flask about 2-3 times;

transferring the suspension from the flask into a conical tube;

adding about 5 mL of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into the flask to rinse and collect residual cells;

transferring the suspension from the flask into the conical tube;

centrifuging the conical tube at about 600xG for about 10 minutes at room temperature resulting in supernatant and pellet;

remove the supernatant, leaving behind approximately about 5 mL;

resuspending the pellet with about 30 mL of complete media;

transferring the suspension into a flask;

incubating the cell suspension in the flask;

(d) cell fraction feeding 3, comprising:

collecting the cell suspension in the flask and dispense it down the bottom of the flask about 2-3 times;

transferring the suspension from the flask into a conical tube;

adding about 5 mL of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into the flask to rinse and collect residual cells;

transferring the suspension from the flask into the conical tube;

centrifuging the conical tube at about 600xG for about 10 minutes at room temperature resulting in supernatant and pellet;

remove the supernatant, leaving behind approximately about 5 mL;

resuspending the pellet with about 30 mL of complete media;

transferring the suspension into a flask;

incubating the cell suspension in the flask.

[0091] In various embodiments, the method further comprises cell fraction harvesting on day of reprogramming.

[0092] In various embodiments, the complete media comprising StemSpan AOF and StemSpan CD34+ Expansion Supplement are an about 8-10:0.5-1.5 ratio of StemSpan AOF:StemSpan CD34+ Expansion Supplement. In various embodiments, the complete media comprising StemSpan AOF and StemSpan CD34+ Expansion Supplement are an about 9:1 ratio of StemSpan AOF:StemSpan CD34+ Expansion Supplement.

[0093] In various embodiments, optionally pooling the cell suspension in the cells tube ("first cells tube") with one or more additional cell suspension into one tube, comprises transferring the cell suspension from the one or more additional cells tubes to the first cells tube; adding complete media into the one or more additional cells tubes to wash and collect residual cells, and transferring the suspension from the one or more additional cells tubes to the first cells tube.

[0094] In various embodiments, optionally pooling the cell suspension in the cells tube ("first cells tube") with one or more additional cell suspension into one tube, comprises transferring the cell suspension from the one or more additional cells tubes to the first cells tube; adding 1 mL of complete media into the one or more additional cells tubes to wash and collect residual cells, and transferring the suspension from the one or more additional cells tubes to the first cells tube.

[0095] In various embodiments, the isolated cells from cord blood sample express one or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22. In various embodiments, the isolated cells from cord blood sample express 5 or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22. In various embodiments, the isolated cells from cord blood sample express 7 or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22. In various embodiments, the isolated cells from cord blood sample express hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22.

[0096] In various embodiments, these isolated cells are the post wash CBU Wash sample. In various embodiments, these isolated cells are the isolated and enriched CBU cells after culturing them for 4 days.

[0097] Various embodiments provide for a cell suspension comprising: isolated cell fraction (ICF) from Cord Blood Units (CBUs); and complete media, wherein complete media comprises Stem-Span AOF and StemSpan CD34+ Expansion Supplement.

[0098] In various embodiments, the isolated cell fraction (ICF) from Cord Blood Units (CBUs) is isolated by any one of the methods of the present invention as described herein.

[0099] In various embodiments, the isolated cells from cord blood sample express one or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22. In various embodiments, the isolated cells from cord blood sample express 5 or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22. In various embodiments, the isolated cells from cord blood sample express 7 or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22. In various embodiments, the isolated cells from cord blood sample express hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22.

[0100] In various embodiments, these isolated cells are the post wash CBU Wash sample. In various embodiments, these isolated cells are the isolated and enriched CBU cells after culturing them for 4 days.

Wash Buffer

[0101] In various embodiments, the Wash Buffer comprises: about 366 mL of DPBS -/- , about 7.5 mL of 25% HSA (to yield a 0.5% solution), and about 1.5 mL of 0.5M EDTA (to yield a 2 mM solution). In various embodiments, the Wash Buffer is in the ratio of about 0.976 DPBS -/- : 0.02 HSA : 0.004 EDTA, each in the aforementioned concentrations.

[0102] In various embodiments, the Wash Buffer is in the ratio of about 0.878-0.996 DPBS -/- : 0.01-0.003 HSA : 0.002-0.006 EDTA, each in the aforementioned concentrations.

Lysis Buffer

[0103] In various embodiments, the Lysis Buffer comprises DNase (4KU) at a 4000U/mL solution. In various embodiments, the Lysis Buffer comprises DNase (4KU) at a 35000-4500U/mL solution.

Complete media

[0104] In various embodiments, the Complete Media comprises StemSpan AOF and StemSpan CD34+ expansion supplement. In various embodiments, the Complete Media comprises StemSpan AOF and StemSpan CD34+ expansion supplement in an about 9:1 ratio. In various embodiments, the Complete Media comprises StemSpan AOF and StemSpan CD34+ expansion supplement in an about 8:1 to 10:1 ratio.

KITS

[0105] The present invention is also directed to a kit for cell wash, isolation and/or cryopreservation. The kit is useful for practicing the inventive method of cell wash, isolation and/or cryopreservation. The kit is an assemblage of materials or components. Thus, in some embodiments the kit contains a composition including the components of the wash buffer or components of the lysis buffer as described above.

[0106] The present invention is also directed to a kit for processing and enrichment of cord blood unit to isolate a viable cell fraction that is capable of being efficiently reprogrammed to iPSCs. The kit is useful for practicing the inventive method of processing and enrichment of cord blood unit isolated cell fractions. The kit is an assemblage of materials or components. Thus, in some embodiments the kit contains a composition including the components of the buffers and cell media as described above.

[0107] The exact nature of the components configured in the inventive kit depends on its intended purpose. For example, some embodiments are configured for the purpose of cell wash. In one embodiment, the kit is configured particularly for the purpose of cell isolation. In another embodiment, the kit is configured particularly for the purpose of cell cryopreservation. In additional examples, some embodiments are configured for the purpose of processing cord blood unit isolated cell fractions. In one embodiment, the kit is configured particularly for the purpose of enrichment of cord blood unit isolated cell fractions.

[0108] Instructions for use may be included in the kit. “Instructions for use” typically include a tangible expression describing the technique to be employed in using the components of the kit to effectuate a desired outcome. Optionally, the kit also contains other useful components, such as, diluents, pharmaceutically acceptable carriers, syringes, catheters, applicators, pipetting or measuring tools, or other useful paraphernalia as will be readily recognized by those of skill in the art.

[0109] The materials or components assembled in the kit can be provided to the practitioner stored in any convenient and suitable ways that preserve their operability and utility. For example, the components can be in dissolved, dehydrated, or lyophilized form; they can be provided at room, refrigerated or frozen temperatures. The components are typically contained in suitable packaging

material(s). As employed herein, the phrase "packaging material" refers to one or more physical structures used to house the contents of the kit, such as inventive compositions and the like. The packaging material is constructed by well-known methods, preferably to provide a sterile, contaminant-free environment. As used herein, the term "package" refers to a suitable solid matrix or material such as glass, plastic, paper, foil, and the like, capable of holding the individual kit components. The packaging material generally has an external label which indicates the contents and/or purpose of the kit and/or its components.

EXAMPLES

[0110] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned or number of items mentioned, it is merely for purposes of illustration and is not intended to limit the invention. One skilled in the art may develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention.

Example 1

Media Preparation

Wash Buffer Preparation

[0111] Transfer the following into a *Wash Buffer* storage bottle and Mix the solution by pipetting up and down.

- 366 mL of DPBS -/-
- 7.5 mL of 25% HSA (to yield a 0.5% solution)
- 1.5 mL of 0.5M EDTA (to yield a 2 mM solution)

Lysis Buffer Preparation

[0112] Fully dissolve a vial of DNase (4KU) by adding 1 mL of water into vial and pipette up and down to ensure it is fully dissolved to yield a 4000U/mL solution.

[0113] Transfer 45 mL of 1X Lysis Buffer into the filter system to filter the Lysis Buffer. After filtering, discard extraneous pieces. Repeat for all three tubes. Transfer 11.25 µl of 4000U/ mL DNase I into each *Lysis Buffer* tube to obtain a working concentration of 1U/ m. Mix the solution by pipetting up and down. Place the *Lysis Buffer* tubes in a 4°C refrigerator until use.

Example 2

CBU Processing

CBU THAW

[0114] Obtain a frozen CBU from LN2 storage, and place on dry ice in the pass-through. Transfer the frozen CBU into a beaker containing sterile water placed inside a 37°C bead bath. Record the thaw start time. Visually inspect the CBU for thawing. Upon thawing confirmation, transfer the CBU into the BSC. Open one seal of the bag with scissors. Attach the fluid transfer set and push in the spike as far in as possible. Remove air from a 60 mL syringe. Attach the syringe to the fluid transfer set. Transfer the total volume of cord blood into a 50 mL conical tube. Repeat the steps for the second portion of the bag, transferring the blood into the same conical tube. After syringing cord blood from both portions of the bag, record the total volume of cord blood collected.

PRE-LYSIS WASH 1

[0115] Label three (3) 50 mL conical tubes as "Cells". Add 33 mL from the "Wash Buffer" into each "Cells" tube. Split the volume of cord blood between the three "Cells" tubes and record volume transferred into each tube. Transfer the three "Cells" tubes into the centrifuge. Centrifuge the tubes at 600xG for 10 minutes at 4 °C.

PRE-LYSIS WASH 2

[0116] After centrifugation, transfer the "Cells" tubes into the BSC. Remove the supernatant from each "Cells" tube. Resuspend each pellet with 30 mL of "Wash Buffer". Transfer the three "Cells" tubes into the centrifuge. Centrifuge the tubes at 600xG for 10 minutes at 4 °C. After centrifugation, transfer the "Cells" tubes into the BSC. Visually inspect the cell pellets for presence of RBCs.

[0117] If there is a clear separation between the pellet and the supernatant – even if the supernatant is red in color – as shown in Figure 1, proceed to the section for Lysis. If the solution is dark red and there is no clearly visible pellet, continue as detailed below to perform a third Pre-Lysis Wash.

PRE-LYSIS WASH 3 (IF APPLICABLE)

[0118] Remove the supernatant from each "Cells" tube and resuspend with 30 mL of solution from the "Wash Buffer" bottle. Transfer, for example, the three "Cells" tubes into the centrifuge. Centrifuge tubes at 600xG for 10 minutes at 4°C. After centrifugation, transfer the "Cells" tubes into the BSC.

LYSIS

[0119] Retrieve the "Lysis Buffer" from the refrigerator. After centrifugation, transfer the "Cells" tubes into the BSC. Remove the supernatant from each "Cells" tube. Resuspend each pellet with 40 mL of "Lysis Buffer". Incubate the "Cells" tubes for 10 minutes at room temperature. Ensure

the cells are not incubated for more than 15 minutes. Transfer the three "Cells" tubes into the centrifuge. Centrifuge the tubes at 600xG for 10 minutes at 4 °C.

POST-LYSIS WASH

[0120] After centrifugation, transfer the "Cells" tubes into the BSC. Remove the supernatant from each "Cells" tube. Resuspend each pellet with 30 mL of solution from the "Wash Buffer" bottle. Transfer the three "Cells" tubes into the centrifuge. Centrifuge the tubes at 600xG for 10 minutes at Room Temperature.

STRAIN

[0121] Label three (3) 50 mL conical tubes as "Strain". After centrifugation, transfer the "Cells" tubes into the BSC. Remove the supernatant. Label each of the "Cells" tubes, respectively, as "Cells 1", "Cells 2", and "Cells 3". Pool the cells into one tube following the steps below:

- Add 5 mL of StemSpan AOF to each of the tubes: "Cells 1", "Cells 2" and "Cells 3". Resuspend each of pellet.
- Transfer the cell suspension from the "Cells 2" tube into the "Cells 1" tube.
- Transfer the cell suspension from the "Cells 3" tube into the "Cells 1" tube, so that "Cells 1" contains the pooled cell suspension.
- Add 7.5 mL of StemSpan AOF into the "Cells 2" tube to wash and collect residual cells. Transfer the suspension from "Cells 2" into the "Cells 1" tube.
- Transfer 7.5 mL of StemSpan AOF into the "Cells 3" tube to collect residual cells. Transfer the suspension from "Cells 3" into the "Cells 1" tube.

[0122] Resuspend any remaining aggregates or clumps by pipetting up and down. Place a 40 µm sterile cell strainer on the "Strain" tube. Transfer 1 mL of StemSpan AOF into the "Strain" tube, coating the surface area of the cell strainer. Gradually transfer the total volume of cell suspension from the "Cells 1" tube into the "Strain" tube through the 40 µm Cell Strainer.

[0123] The strainer may become overburdened and not allow suspension to pass through. Change the cell strainer as and when necessary. Ensure to coat the strainer before straining the cell suspension as detailed in 7.4.7.6.

[0124] After straining the entire volume of cell suspension, record the total volume of cell suspension (A) in the Strain tube.

CELL COUNT

[0125] Label two (2) 1.5 mL microcentrifuge tubes as "Dil." and "Count". Transfer 900 µl of StemSpan AOF to the "Dil." microcentrifuge tube. Transfer 100 µl of well-mixed cell suspension from the Strain tube into the "Dil." microcentrifuge tube to create a 1:10 dilution. Pipette up and down to

mix. Transfer 135 µl of Moxi Cyte dye into the "Count" microcentrifuge tube. Transfer 15 µl of cell suspension from the "Dil." tube into the "Count" microcentrifuge tube, creating a 1:100 dilution. Transfer the "Count" microcentrifuge tube outside of the BSC. Incubate the "Count" microcentrifuge tube for 5 minutes at room temperature in the dark. After 5 minutes of incubation time, count the cells using the Moxi Go II . Report the Viability and Live Count (cells/ mL) in the respective fields on the BPR. Calculate the average Viability and Average Live Count (B).

[0126] Calculate the average cell concentration in the "Strain" tube using the Average live count in the table below.

Dilution factor		Average Live Count		"Strain" Tube Cell Density
100	×	B	=	C

[0127] Calculate the Total Viable Cells using the Suspension Volume (A from step 7.4.7.8) and the "Strain" Tube Cell Density (C from the calculation in step 7.5.9) in the table below.

Suspension Volume (mL)		"Strain" Tube Cell Density		Total Viable Cells
A	×	C	=	D

SAMPLING

[0128] Use the "Strain" Tube Cell Density (C from the about calculation) to calculate the volume of cell suspension required to obtain 4E6 cells for two cell pellet samples in the table below. NOTE: If the target volume is too small, pull a larger, workable volume and split it evenly between two microcentrifuge tubes.

Cells Required for Sampling (cells)		"Strain" Tube Cell Density (cells/ mL)		Target Volume for SAMPLE: Cell Pellet (mL)
2E6	÷	C	=	E

[0129] Transfer the volume calculated for Cell Pellet (E from the calculation in above) into two microcentrifuge tubes. Transfer them out of the BSC for centrifugation. Using a microcentrifuge tube adapter, transfer the 2 cell pellet tubes and the "Strain" tube into the centrifuge. Centrifuge at 600xG for 10 minutes at 4°C. While the "Strain" tube and the microcentrifuge tubes are the centrifuge, determine the Total Viable Cells Remaining using the number of Total Viable Cells (D from the calculation in above).

Total Viable Cells		Number of Cells for Cell Pellet Sampling (cells/ mL)		Total Viable Cells Remaining
D	-	4E6	=	E

[0130] While the "Strain" tube and the microcentrifuge tubes are in the centrifuge, determine the Target CS10 Volume using the Total Viable Cells Remaining (E from the calculation above).

Total Viable Cells Remaining		Vial Cell Density (cells/ mL)		Target CS10 Volume (mL)
E	÷	5E6	=	F

[0131] While the "Strain" tube and the microcentrifuge tubes are in the centrifuge, determine the designated freezing container. For target CS10 volumes less than 50 mL, use a 50 mL conical tube. For volumes greater than 50 mL, use a storage bottle. Record container used.

[0132] While the "Strain" tube and the microcentrifuge tubes are in the centrifuge, determine the Additional CS10 Volume Required following resuspension using the Target CS10 Volume (F from the calculation above).

Target CS10 Volume (mL)		CS10 Added (mL)		Additional CS10 Volume (mL)
F	-	20	=	G

[0133] While the "Strain" tube and the microcentrifuge tubes are in the centrifuge, label the designated freeze container as "Freeze Stock". Label a 15 mL conical tube as "QC".

[0134] While the "Strain" tube and the microcentrifuge tubes are in the centrifuge, prepare a 5 mL "Temperature Probe" vial.

[0135] Transfer 4.5 mL of CS10 into the 5 mL vial. Transfer the "Temperature Probe" vial out of the BSC for labeling.

[0136] Upon completion, transfer all tubes back into the BSC from the centrifuge.

[0137] Using a micropipette, carefully remove the supernatant, ensuring not to disturb the cell pellet. Once complete, transfer both microcentrifuge tubes out of the BSC for labelling.

Final Formulation and Cryopreservation

[0138] After centrifugation, discard the supernatant from the "Strain" tube. Add 5 mL of CS10 to break up the cell pellet. Transfer an additional 15 mL CS10 to the "Strain" tube. Pipette up and down

to mix. Transfer the cell suspension to the "Freeze Stock" container. Add the Additional CS10 Volume (G from above) needed to yield the target CS10 Volume (F from above) to the "Freeze Stock" container. Record the time of CS10 addition.

[0139] To obtain 16E6 cells for QC, transfer 3.2 mL of cell suspension from the "Freeze Stock" container into the "QC" tube. Add 4.8 mL of CS10 into the "QC tube" to yield 2E6 cells/ mL. Transfer 1 mL of cell suspension from the "QC" tube into 2 mL cryovials. Repeat to achieve eight (8) QC cryovials with 1 mL of suspension. Transfer the QC vials outside of the BSC for labeling.

[0140] Transfer 4.5 mL of cell suspension from the "Freeze Stock" container into 5 mL cryovials. Transfer the product vials outside of the BSC for labeling.

CRF AND STORAGE

[0141] Set up the CRF using the protocol below.

Steps	**Rate (°C/min)**	**End Temperature (°C)**
1	Hold	4.0
2	1.00 (sample)	−4.0
3	25.00 (chamber)	−40.0
4	10.00 (chamber)	−12.0
5	1.00 (chamber)	−40.0
6	10.00 (chamber)	−90.0
7	End	N/A

[0142] Load the CRF with all vials. Place the CRF probe in the TEMP PROBE vial 7.9.3 Confirm the CRF protocol and record the CRF start time. Upon completion, record the CRF end time. Transfer the vials into LN2 storage.

Example 3

[0143] This process described herein is typically performed by thawing a cell fraction previously isolated from a Cord Blood Unit (CBU).

[0144] This culture is performed prior to reprogramming to iPSCs. As such, culture days are numbered counting down to the day of reprogramming – referred to as Day-0, as noted herein: Cell Fraction Thaw and Seed = Day -4, Cell Fraction Feed 1 = Day -3, Cell Fraction Feed 2 = Day -2, Cell Fraction Feed 3 = Day -1, Cell Fraction Harvest = Day 0

DAY - 4 PROCEDURE

[0145] Obtain StemSpan AOF and StemSpan CD34+ Expansion Supplement (10X). Place the media and supplement at room temperature for at least 30 minutes. Record the equilibration start time and end times.

Complete Media Compounding

[0146] Transfer 36 mL of Stem-Span AOF into each "Complete Media" tube. Transfer 4 mL of StemSpan CD34+ Expansion Supplement into each "Complete Media" tube.

Vial Thaw and Resuspension.

[0147] Determine the number of vials of Isolated Cell Fraction to thaw. The sequence of priorities is as follows:

[0148] Cells will be cultured in two (2) T175 flasks at a density ranging from 1.3E5 cells/cm^2 – 3.25E5 cells/cm^2. This is equal to 4.55E7 – 1.14E8 total cells between the two flasks.

[0149] Enough vials will be thawed to culture cells at the highest density possible, on condition that no more than half the total number of 5 mL product vials are thawed. This will allow for enough cells to repeat the process one time, if necessary.

[0150] BPR will calculate number of vials required to yield the highest seeding density. Based on current inventory, user will enter highest number of vials available (based on priority sequence described above) and will confirm that total expected cell number falls within acceptable range.

[0151] Vials will be thawed to culture cells at the highest density possible, on condition that no more than half the total number of 5 mL product vials are thawed. This will allow for enough cells to repeat the process one time, if necessary.

[0152] The post-thaw cell count and viability results reported as per release for the CBU ICF vials will be used to estimate how many vials to thaw based on the expected post-thaw recovery.

[0153] High Density Case: If the expected post-thaw recovery is 35% and there are at least 6.5E8 CBU ICF cells in stock, half the cells (3.25E8) will be thawed. This will yield 1.14E8 cells to be cultured (3.25E8 × 35% = 1.14E8), allowing operator to seed cells at highest density and still be left with half the number of vials for an additional run.

[0154] Mid Density Case: If there are less than 6.5E8 CBU ICF cells in stock (which is enough for the highest density case), but more than 2.6E8 Cells (which is the minimum required to seed the low-density case), the density will be adjusted accordingly to equally seed two (2) T175 flasks. This too will allow for enough vials for an additional run.

[0155] In the event there are less than 2.6E8 CBU ICF Cells in stock, consult with the Manufacturing Manager and Client to determine best course of action for processing.

[0156] Based on the number of vials to thaw, label the corresponding number of 50 mL conical tubes as "Cells". In addition, label two microcentrifuge tubes as "Count Sample (CS)" and "Moxi".

[0157] Obtain the required number of frozen vials from LN2 storage. Record the number of vials and the vial label information.

[0158] Perform the following steps with a maximum of 4 cryovials at a time. As such, group the maximum number of vials as seen in Figure 2. Record the number of vial thaw groups required.

[0159] Label the four (4) 50 mL conical tubes as "Cells X.1", "Cells X.2", "Cells X.3", "Cells X.4", where "X" corresponds to the vial thaw group number. There may be a maximum of 4 vials in each group, as seen in Figure 3.

[0160] Transfer 4.5 mL of StemSpan AOF into the tubes labeled "Cells X.1", "Cells X.2", "Cells X.3", and "Cells X.4". Transfer the frozen vials into a 37°C beaker in the bead bath. Record the thaw start time. Visually inspect the vials for thawing. Record the thaw end time. Transfer the vials into the BSC. Transfer vial contents of one vial into one "Cells" tube. Repeat for the remaining vials, transferring one vial contents into a respective tube as seen in Figure 4.

[0161] Use 4.5 mL of StemSpan AOF to rinse each tube and collect residual cells. Transfer the suspension into the respective "Cells" tube. Transfer an additional 31.5 mL of StemSpan AOF into each "Cells" tube to yield a total of 45ml. Transfer the tubes outside of the BSC. Transfer all of the "Cells" tubes into the centrifuge. Centrifuge the tubes at 600xG for 10 minutes at room temperature. After centrifugation, transfer the "Cells" tubes back into the BSC. Discard the supernatant. Resuspend each pellet with 1 mL of Complete Media. Transfer the suspensions from "Cells X.2", "Cells X.3" and "Cells X.4" into the "Cells X.1" tube, as seen in Figure 5. Approximately 4 mL of cell suspension should be in "Cells X.1".

[0162] Rinse the tubes by transferring 1 mL of Complete Media into the "Cells X.2", "Cells X.3", and "Cells X.4" tubes.

[0163] After rinsing, pool all the suspension into Cells X.1, as seen in Figure 6.

[0164] The "Cells X.2", "Cells X.3", and "Cells X.4" from this vial thaw group may be discarded.

[0165] Repeat the steps for the remaining vial thaw groups, thawing no more than four (4) 5 mL vials at a time. For each group of cryovials, label them with an increased number for "X". For example, thawing group 2 may contain "Cells 2.1", "Cells 2.2", "Cells 2.3", and "Cells 2.4"., while thawing group 3 may contain "Cells 3.1", "Cells 3.2", "Cells 3.3", and "Cells 3.4".

[0166] After resuspending all of the thawing groups, each "Cells X.1" tube will contain approximately 7 mL of cell suspension. Once all the vials have been thawed, transfer all the cell suspensions in the "Cells X.1" vials into "Cells 1.1" as seen in Figure 7.

[0167] Measure the total volume of cell suspension (A) in the "Cells 1.1" tube using a serological pipette.

Count

[0168] Transfer 1 mL of well mixed cell suspension from the "Cells 1.1" tube into the "Count Sample (CS)" microcentrifuge tube. Transfer 135 µL of Moxi Cyte dye into the "Moxi" microcentrifuge tube. Transfer 15 µL of cell suspension from the "Count Sample (CS)" microcentrifuge tube into the "Moxi" microcentrifuge tube, creating a 1:10 dilution. Transfer the "Moxi" microcentrifuge tube outside of the BSC. Incubate the "Moxi" microcentrifuge tube for 5 minutes at room temperature in the dark.

[0169] After 5 minutes of incubation time, count the cells, for example, using the Moxi Go II. Report the Viability and Live Count (cells/ mL) in the respective fields on the BPR.

[0170] Calculate the average Viability and average Live Count (B). Calculate the average cell concentration in the "Cells 1.1" tube (C) using the average live count (B from above).

Dilution factor		Average Live Count		"Cells 1.1" Cell Concentration
10	×	B	=	C

[0171] Calculate the total number of cells obtained (D) using the suspension volume (A from 8.5.10) and the "Cells 1.1 Cell Concentration" (C above).

Suspension Volume		"Cells 1.1" Cell Concentration		Total Number of Cells Obtained
A	×	C	=	D

Day - 4 Seed

[0172] Confirm that the total number of cells obtained (D from step 8.6.8) is within the range of 4.55E7 – 1.14E8 cells, which was previously described above. If within the correct range, the total volume of cell suspension will be split equally between the two (2) T175 flasks. If total number of cells is outside the range, notify manufacturing manager or supervisor for further instruction.

[0173] Calculate the volume of cell suspension (E) to transfer into each flask using the Suspension Volume (A from 8.5.10)

Total Volume in Cells 1.1 (mL)		Number of Flasks		Volume of Cell Suspension Per Flask (mL)
A	÷	2	=	E

[0174] Label two (2) T175s with the information such as Sample ID, BPR#, Flask number (either 1 or 2), Day - 4 (D -4), Initials, Date.

[0175] Determine the additional volume of "Complete Media" required for each T175 flask to contain 35 mL using the Volume of Cell Suspension per Flask (E from above).

Volume per Flask (mL)		Volume of Cell Suspension per Flask (ml)		Required Complete Medial per Flask (mL)
35	-	E	=	F

[0176] Transfer the calculated volume of Complete Media (F from above) into each flask. Next, transfer the calculated volume of cell suspension (E from above)

[0177] Place the flasks in an incubator. Record the Day -4 incubation start time. Record Day -4 end date and end time.

DAY -3 PROCEDURE

[0178] Obtain StemSpan AOF and StemSpan CD34+ Expansion Supplement (10X). Place the media and supplement at room temperature for 30 minutes. Record the equilibration start time.

Complete Media Compounding

[0179] Record the StemSpan AOF and StemSpan CD34+ expansion supplement end equilibration times.

[0180] Transfer 36 mL of StemSpan AOF into each "Complete Media" tube. Transfer 4 mL of StemSpan CD34+ Expansion Supplement into each "Complete Media" tube.

Day -3 Feed

[0181] Remove the Day -4 flasks from the incubator. Record the flask removal time. Visually inspect the flasks under a microscope and take a picture. Label the photo with the following information such as Project name, BPR#, CBU Unique ID (PROJECT-XXXX), Flask number (either flask 1 or flask 2), Day -3 (D-3), Initials and date

[0182] Transfer the Day -4 flasks into the BSC. Label two (2) 50 mL conical tubes as "#1" and "#2".

[0183] Collect the cell suspension in "Flask #1" and dispense it down the bottom of the flask 2-3 times. Next, transfer the suspension from "Flask #1" into the "#1" tube.

[0184] Transfer 5 mL of "Complete Media" into "Flask 1" to rinse and collect residual cells. Transfer the suspension from "Flask 1" into the "#1" tube.

[0185] Collect the cell suspension in "Flask #2" and dispense it down the bottom of the flask 2-3 times. Next, transfer the suspension from the "Flask #2" into the "#2" tube.

[0186] Transfer 5mL of "Complete Media into the "Flask 2" to rinse and collect residual cells. Transfer the suspension from "Flask 2" into the "#2" tube.

[0187] Transfer the "#1" and "#2" tubes out of the BSC. Transfer the tubes labeled #1 and #2 into the centrifuge. Centrifuge the tubes at 600xG for 10 minutes at room temperature.

[0188] During centrifugation, the Day -4 "Flask 1" and "Flask 2" T175s may be discarded

[0189] During centrifugation, transfer two (2) new T175 flasks into the BSC. Label the flasks with the information such as: Sample ID, BPR#, Flask number (either flask 1 or flask 2), Day -3 (D-3), Initials and Date.

[0190] After centrifugation, transfer the "#1" and "#2" tubes into the BSC. Remove the supernatant, leaving behind approximately 5 mL. Resuspend the pellet with an additional 30 mL of StemSpan Complete Media. Pipette up and down to mix. Transfer the suspension from "Tube #1" into "Flask #1". 9.4.15 Transfer the suspension from "Tube #2" into "Flask #2". Transfer both flasks into the incubator. Record the Day -3 incubation start time. Record Day -3 end date and end time.

DAY -2 PROCEDURE

[0191] Obtain StemSpan AOF and StemSpan CD34+ Expansion Supplement (10X). Place the media and supplement at room temperature for 30 minutes. Record the equilibration start time.

Complete Media Compounding

[0192] Record the StemSpan AOF and StemSpan CD34+ expansion supplement end equilibration times. Transfer 36 mL of StemSpan AOF into each "Complete Media" tube. Transfer 4 mL of StemSpan CD34+ Expansion Supplement into each "Complete Media" tube.

Day -2 Feed

[0193] Remove the Day -3 flasks from the incubator. Record the flask removal time.

[0194] Visually inspect the flasks under a microscope and take a picture. Label the photo with the information such as: Project name, BPR#, CBU Unique ID (PROJECT-XXXX), Flask number (either flask 1 or flask 2), Day -2 (D-2), Initials and date.

[0195] Transfer the Day -3 flasks into the BSC. Label two (2) 50 mL conical tubes as "#1" and "#2". Collect the cell suspension in "Flask #1" and dispense it down the bottom of the flask 2-3 times. Next, transfer the suspension from "Flask #1" into the "#1" tube.

[0196] Transfer 5 mL of "Complete Media" into "Flask 1" to rinse and collect residual cells. Transfer the suspension from "Flask 1" into the "#1" tube. Collect the cell suspension in "Flask #2" and dispense it down the bottom of the flask 2-3 times. Next, transfer the suspension from the "Flask #2" into the "#2" tube.

[0197] Transfer 5mL of "Complete Media into the "Flask 2" to rinse and collect residual cells. Transfer the suspension from "Flask 2" into the "#2" tube. Transfer the "#1" and "#2" tubes out of the BSC.. Transfer the tubes labeled #1 and #2 into the centrifuge. Centrifuge the tubes at 600xG for 10 minutes at room temperature.

[0198] During centrifugation, the Day -3 "Flask 1" and "Flask 2" T175s may be discarded. During centrifugation, transfer two (2) new T175 flasks into the BSC.

[0199] Label the flasks with the information such as: Sample ID, BPR#, Flask number (either flask 1 or flask 2), Day -2 (D-2), Initials and Date.

[0200] After centrifugation, transfer the "#1" and "#2" tubes into the BSC. Remove the supernatant, leaving behind approximately 5 mL. Resuspend the pellet with an additional 30 mL of StemSpan Complete Media. Pipette up and down to mix.

[0201] Transfer the suspension from "Tube #1" into "Flask #1". Transfer the suspension from "Tube #2" into "Flask #2". Transfer both flasks into the incubator. Record the Day -2 incubation start time.

DAY -1 PROCEDURE

[0202] Obtain StemSpan AOF and StemSpan CD34+ Expansion Supplement (10X). Place the media and supplement at room temperature for 30 minutes. Record the equilibration start time.

Complete Media Compounding

[0203] Record the StemSpan AOF and StemSpan CD34+ expansion supplement end equilibration times. Transfer 36 mL of StemSpan AOF into each "Complete Media" tube.

Transfer 4 mL of StemSpan CD34+ Expansion Supplement into each "Complete Media" tube.

Day -1 Feed

[0204] Remove the Day -2 flasks from the incubator. Record the flask removal time. Visually inspect the flasks under a microscope and take a picture. Label the photo with the information such as: Project name, BPR#, CBU Unique ID (PROJECT-XXXX), Flask number (either flask 1 or flask 2), Day -1 (D-1), Initials and date.

[0205] Transfer the Day -2 flasks into the BSC. Label two (2) 50 mL conical tubes as "#1" and "#2". Collect the cell suspension in "Flask #1" and dispense it down the bottom of the flask 2-3 times. Next, transfer the suspension from "Flask #1" into the "#1" tube. Transfer 5 mL of "Complete Media" into "Flask 1" to rinse and collect residual cells. Transfer the suspension from "Flask 1" into the "#1" tube. Collect the cell suspension in "Flask #2" and dispense it down the bottom of the flask 2-3 times. Next, transfer the suspension from the "Flask #2" into the "#2" tube. Transfer 5mL of "Complete Media into the "Flask 2" to rinse and collect residual cells. Transfer the suspension from "Flask 2" into the "#2" tube. Transfer the "#1" and "#2" tubes out of the BSC. Transfer the tubes labeled #1 and #2 into the centrifuge. Centrifuge the tubes at 600xG for 10 minutes at room temperature.

[0206] During centrifugation, the Day -2 "Flask 1" and "Flask 2" T175s may be discarded. During centrifugation, transfer two (2) new T175 flasks into the BSC. Label the flasks with the

information such as: Sample ID, BPR#, Flask number (either flask 1 or flask 2), Day -1 (D-1), Initials and Date.

[0207] After centrifugation, transfer the "#1" and "#2" tubes into the BSC. Remove the supernatant, leaving behind approximately 5 mL. Resuspend the pellet with an additional 30 mL of StemSpan Complete Media. Pipette up and down to mix. Transfer the suspension from "Tube #1" into "Flask #1". 11.4.15 Transfer the suspension from "Tube #2" into "Flask #2". Transfer both flasks into the incubator. Record Day -1 incubation start time.

DAY 0 PROCEDURE

[0208] Cells will be harvested for reprogramming.

Example 7

Single cell RNA-sequencing

[0209] Single-cell RNA sequencing (scRNA-seq) is a powerful technology that enables the measurement of transcriptomes at the resolution of individual cells. It is a powerful technique that allows researchers to analyze gene expression profiles at the individual cell level. This method provides unprecedented resolution for understanding cellular heterogeneity, identifying rare cell populations, and tracking cellular differentiation processes.

[0210] We used the 10X Genomics Chromium system, which you used, employs a droplet-based approach for scRNA-seq. Here is a brief overview of the process:

- Single cells are encapsulated in Gel Beads in Emulsion (GEMs) along with barcoded oligonucleotides.
- Within each GEM, cell lysis occurs, and mRNA is captured and barcoded.
- The barcoded cDNA is amplified and used to prepare sequencing libraries.
- Libraries are sequenced using next-generation sequencing platforms.

[0211] Described herein is a brief overview of the process and how it is used to identify gene markers and cluster cells using methods like UMAP, particularly in the context of the 10x Genomics platform:

[0212] **Single-Cell Isolation and Library Preparation (10x Genomics):** Thousands of individual cells are encapsulated into microdroplets along with uniquely barcoded beads. Each bead carries a unique oligonucleotide barcode. Within each droplet, polyadenylated mRNA from a single cell is captured on the barcoded bead, followed by reverse transcription to generate cDNA. This barcode identifies all transcripts that originate from the same cell. After breaking the emulsion, the cDNA is

amplified, and sequencing libraries are prepared. Each resulting library contains transcript information linked to an individual cell.

[0213] **Sequencing and Data Processing:** Libraries are sequenced, typically on Illumina platforms. Raw reads are demultiplexed using the 10x Genomics Cell Ranger pipeline, which aligns reads to a reference genome, quantifies gene expression, and outputs a cell-by-gene expression matrix.

[0214] **Identifying Marker Genes:** Expression data are normalized to account for differences in sequencing depth and other technical variations. Marker genes for clusters or specific cell types are identified by comparing the expression profiles of groups of cells (e.g., cluster 1 vs. all other cells). After sequencing, the data is processed to generate a gene expression matrix for each cell. This matrix is then used to identify marker genes that characterize different cell populations. High expression (and specificity) of a gene within a cluster suggests it may be a marker for that cell population. Known markers can help assign identities to clusters (e.g., T cells, neurons, epithelial cells, etc.), while novel markers may suggest new cell subtypes or states. Differential expression analysis is performed between clusters or cell types. Genes that are significantly upregulated in a specific cluster compared to others are considered potential markers. The FindAllMarkers() function in Seurat or Loupe Browser, that are popular packages for scRNA-seq analysis

[0215] **Clustering and UMAP Analysis**: Dimensionality reduction is used. Uniform Manifold Approximation and Projection (UMAP) is a dimensionality reduction technique widely used for visualizing and clustering scRNA-seq data. Given that the data can include thousands of genes and thousands of cells, techniques like principal component analysis (PCA) followed by Uniform Manifold Approximation and Projection (UMAP) are used to project the high-dimensional data into a lower-dimensional space. UMAP creates a two-dimensional (or sometimes three-dimensional) representation that preserves local and global similarities between cells. Cells with similar expression profiles cluster together in this reduced space. The UMAP plot is used to visualize how cells group based on their gene expression, and each cluster can be annotated based on known or newly This allows for visualization of cellular relationships and identification of distinct cell populations.

[0216] Single cell RNA-sequencing (sc-RNA seq) was performed on sample data sets from cord blood post wash, cord blood post enrichment (isolated cell fraction), reprogrammed mix of cells and isolated clones of bona-fide iPSCs that were expanded (cord blood-derived).

[0217] Reprogrammed mix are cord blood isolated cell fraction cells exposed to iPSC reprogramming factors and collected at about 16 to 30 days post reprogramming. They are cells that are either completely reprogrammed, partially reprogrammed or unreprogrammed cells towards the iPS cell fate.

[0218] iPSCs that have been expanded for 4 to 6 passages were characterized by scRNA seq. These iPSCs are at about day 45 to day 65 post reprogramming; and 28 to 42 days post clone isolation.

[0219] UMAP clustering of single cell data separates distinct sample sets and distinct clusters are found across samples. Dot plot of markers across clusters are show in Figure 10.

[0220] Known blood and hematopoietic stem/ progenitor marker genes expressed include but are not limited to CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, and PTPN22. (See figures 12-16).

[0221] CD genes expressed in the iPSCs include but are not limited to CD15, CD13, CD133, CD135, CD90, CD117, CD56, CD71, CD10, CD24, CD9, CD49d, CD44, CD71, CD138, KITLG (ligand for KIT/CD117), CD74, and CD326. (Data not shown.)

[0222] Novel genes expressed uniquely in iPSCs include but are not limited to TCFP2L1, CD10, FOXD3, MIR1915HG, IDO1, PRDM14, GRID2, HHLA1, TRDN, C9ORF135, CLDN7, RAB17, APELA, SERPINB9, FLT1 (VEGFR1), GRPR, CXCL5, CXCL12, CUZD1, and COBL. (Data not shown.)

[0223] Known pluripotency genes expressed in the various data sets include but are not limited to GDF3, POU5F1B, DNMT3B, ZIC3, SOX2, NANOGP8, DPPA2, DPPA4, ZFP42, and PODXL (CD34 sialomucin). (Data not shown.)

[0224] Various embodiments of the invention are described above in the Detailed Description. While these descriptions directly describe the above embodiments, it is understood that those skilled in the art may conceive modifications and/or variations to the specific embodiments shown and described herein. Any such modifications or variations that fall within the purview of this description are intended to be included therein as well. Unless specifically noted, it is the intention of the inventors that the words and phrases in the specification and claims be given the ordinary and accustomed meanings to those of ordinary skill in the applicable art(s).

[0225] The foregoing description of various embodiments of the invention known to the applicant at this time of filing the application has been presented and is intended for the purposes of illustration and description. The present description is not intended to be exhaustive nor limit the invention to the precise form disclosed and many modifications and variations are possible in the light of the above teachings. The embodiments described serve to explain the principles of the invention and its practical application and to enable others skilled in the art to utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. Therefore,

it is intended that the invention is not limited to the particular embodiments disclosed for carrying out the invention.

[0226] While particular embodiments of the present invention have been shown and described, it will be obvious to those skilled in the art that, based upon the teachings herein, changes and modifications may be made without departing from this invention and its broader aspects and, therefore, the appended claims are to encompass within their scope all such changes and modifications as are within the true spirit and scope of this invention. As used herein the term "comprising" or "comprises" is used in reference to compositions, methods, and respective component(s) thereof, that are useful to an embodiment, yet open to the inclusion of unspecified elements, whether useful or not. It will be understood by those within the art that, in general, terms used herein are generally intended as "open" terms (e.g., the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.). Although the open-ended term "comprising," as a synonym of terms such as including, containing, or having, is used herein to describe and claim the invention, the present invention, or embodiments thereof, may alternatively be described using alternative terms such as "consisting of" or "consisting essentially of."

[0227] Unless stated otherwise, the terms "a" and "an" and "the" and similar references used in the context of describing a particular embodiment of the application (especially in the context of claims) may be construed to cover both the singular and the plural. The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein may be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (for example, "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the application and does not pose a limitation on the scope of the application otherwise claimed. The abbreviation, "e.g." is derived from the Latin exempli gratia, and is used herein to indicate a non-limiting example. Thus, the abbreviation "e.g." is synonymous with the term "for example." No language in the specification should be construed as indicating any non-claimed element essential to the practice of the application.

[0228] "Optional" or "optionally" means that the subsequently described circumstance may or may not occur, so that the description includes instances where the circumstance occurs and instances where it does not.

[0229] Groupings of alternative elements or embodiments of the present disclosure disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed

individually or in any combination with other members of the group or other elements found herein. One or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is herein deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of washing a frozen cord blood sample and isolating cells, comprising

(a) thawing a frozen cord blood unit (CBU), comprising:

obtaining CBU;

transferring the frozen CBU into a container containing sterile water placed in a 35°C-39°C bath to thaw into cord blood;

(b)(i) performing a first prelysis wash, comprising:

transferring the cord blood into a first tube;

transferring at least a portion the cord blood into a second tube containing wash buffer ("cells tube");

centrifuging the cells tube at 500xG-1000xG for 8-12 minutes at 4°C-20°C resulting in supernatant and pellet;

(b)(ii) performing a second prelysis wash, comprising

removing the supernatant from the centrifuged cells tube;

resuspending the pellet with 15-45 mL of wash buffer;

centrifuging the cells tube containing the resuspended pellet at 500xG-1000xG for 8-12 minutes at 4°C-20°C resulting in supernatant and pellet;

(b)(iii) optionally, performing a third prelysis wash, comprising:

removing the supernatant from the centrifuged cells tube;

resuspending the pellet with 30mL of wash buffer;

centrifuging the cells tube containing the resuspended pellet at 500xG-1000xG for 8-12 minutes at 4°C-20°C resulting in supernatant and pellet;

(c) performing lysis of red blood cells (RBC), comprising:

removing the supernatant from the cells tube;

resuspending the pellet with 20-60 mL of lysis buffer;

incubating the cells tube for up to 15 minutes at room temperature;

centrifuging the cells tube at 500xG-1000xG for 8-12 minutes at 4°C-20°C resulting in supernatant and pellet;

(d) performing a post lysis wash, comprising:

removing the supernatant from the cells tube;

resuspending the pellet with 15-45 mL of wash buffer;

centrifuging the cells tube at 500xG-1000xG for 8-12 minutes at 15-25°C resulting in supernatant and pellet;

(e) straining the cells, comprising:

removing the supernatant from the cells tube;

adding StemSpan AOF to the cells tube;

resuspending the pellet into a cell suspension;

optionally, pooling the cell suspension in the cells tube with one or more additional cell suspension into one tube;

transferring StemSpan AOF into a third tube ("strain tube") through a 30µm-100µm sterile cell strainer, coating the surface area of the cell strainer;

transferring the cell suspension from the cells tube through the cell strainer into the strain tube.

2. The method of claim 1, wherein the method comprises

(a) thawing a frozen cord blood unit (CBU), comprising:

obtaining CBU;

transferring the frozen CBU into a container containing sterile water placed in an about 37°C bath to thaw into cord blood;

(b)(i) performing a first prelysis wash, comprising:

transferring the cord blood into a first conical tube;

transferring at least a portion the cord blood into a second conical tube containing wash buffer ("cells tube");

centrifuging the cells tube at about 600xG for 10 minutes at about 4°C resulting in supernatant and pellet;

(b)(ii) performing a second prelysis wash, comprising

removing the supernatant from the centrifuged cells tube;

resuspending the pellet with about 30mL of wash buffer;

centrifuging the cells tube containing the resuspended pellet at about 600xG for about 10 minutes at about 4°C resulting in supernatant and pellet;

(b)(iii) optionally, performing a third prelysis wash, comprising:

removing the supernatant from the centrifuged cells tube;

resuspending the pellet with about 30mL of wash buffer;

centrifuging the cells tube containing the resuspended pellet at about 600xG for about 10 minutes at about 4°C resulting in supernatant and pellet;

(c) performing lysis of red blood cells (RBCs), comprising:

removing the supernatant from the cells tube;

resuspending the pellet with about 40mL of lysis buffer;

incubating the cells tube for about 10-15 minutes room temperature;

centrifuging the cells tube at about 600xG for about 10 minutes at about 4°C resulting in supernatant and pellet;

(d) performing a post lysis wash, comprising:

removing the supernatant from the cells tube;

resuspending the pellet with about 30 mL of wash buffer;

centrifuging the cells tube at about 600xG for about 10 minutes at about room temperature resulting in supernatant and pellet;

(e) straining the cells, comprising:

removing the supernatant from the cells tube;

adding about 5mL of StemSpan AOF to the cells tube;

resuspending the pellet into a cell suspension;

optionally, pooling the cell suspension in the cells tube with one or more additional cell suspension into one tube;

transferring about 1mL of StemSpan AOF into a third conical tube ("strain tube") through an about 40 μm sterile cell strainer, coating the surface area of the cell strainer;

transferring the cell suspension from the cells tube through the cell strainer into the strain tube.

3. The method of claims 1 or 2, wherein the first tube, second tube, and/or third tube is a conical tube.

4. The method of claims 1 or 2, further comprising transferring, under aseptic conditions, the thawed CBU into a biological safety cabinet (BSC).

5. The method of claims 1 or 2, wherein pooling the cell suspension in the cells tube ("first cells tube") with one or more additional cell suspension into one tubes, comprises:

adding about 5 ml of StemSpan AOF to the first cells tube and to one or more additional cells tube;

resuspending the pellet in the first cells tube and resuspending the pellet in the one or more additional cells tubes;

transferring the cell suspension from the one or more additional cells tubes to the first cells tube;

adding about 7.5 mL of StemSpan AOF into the one or more additional cells tubes to wash and collect residual cells, and transferring the suspension from the one or more additional cells tubes to the first cells tube; and

optionally, resuspending any remaining aggregates or clumps.

6. The method of any one of claims 1-5, further comprising adding cryopreservation solution to the strain tube for cryopreservation.
7. The method of claim 6, further comprising cryopreserving the cells.
8. The method of claims 1 or 2 , wherein the wash buffer comprises DPBS:HSA:EDTA ratio of 366:7.5:1.5, the concentrations of HSA and EDTA are 0.5% HSA and 2 mM EDTA.
9. The method of claims 1 or 2 , wherein the concentration of the lysis buffer is about 4000U/mL of DNase (4KU).
10. The method of claims 1 or 2, wherein the lysis buffer is filtered and results in a concentration of about 1U/mL.
11. A cell suspension comprising:

isolated cells from cord blood sample; and

StemSpan AOF.
12. The cell suspension of claim 11, wherein the isolated cells from cord blood sample is isolated by any one of the methods of claims 1-10.
13. A method of automated filling and cryopreserving isolated cells from cord blood sample , comprising:

(a) obtaining isolated cells from cord blood sample, wherein the isolated cells from cord blood sample have been spun down in a tube;

(b) removing supernatant from pellet;

(c) resuspending the pellet;

(d) adding CS10 to the tube and resuspending the pellet into an isolated cells from cord blood sample suspension;

(e) optionally, combing the isolated cells from cord blood sample suspension with one or more isolated cells from cord blood sample suspensions;

(f) adding additional CS10 to bring the volume of the isolated cells from cord blood sample suspension to 15-35 mL;

(g) transferring the isolated cells from cord blood sample suspension to a 125-375 mL sterile bottle;

(h) adding additional CS10 to bring the volume of the isolated cells from cord blood sample suspension to 100-200 mL;

(i) inserting a tube into the bottle comprising the isolated cells from cord blood sample suspension and priming the tubing until some isolated cells from cord blood sample suspension is dispensed into a reservoir;

(j) placing a multi-vial rack on a rack nest; and

(k) selecting a desired dispensing volume on an automated filling machine;

(l) operating the automated filling machine to fill the vials in the multi-vial rack; and

(m) transferring the filled cryovial to the controlled rate freezer (CRF).

14. The method of claim 13, wherein automated filling and cryopreserving isolated cells from cord blood sample, comprises:

(a) obtaining harvested isolated cells from cord blood sample, wherein the isolated cells from cord blood sample have been spun down in a tube;

(b) removing supernatant from pellet using a serological pipette;

(c) resuspending the pellet by flicking the tube;

(d) adding about 1mL of CS10 to the tube and pipette up and down no more than 3 times to break up the pellet resulting in an isolated cells from cord blood sample suspension ;

(e) optionally, combing the isolated cells from cord blood sample suspension with one or more iPSC suspensions;

(f) adding additional CS10 to bring the volume of the isolated cells from cord blood sample suspension to about 25 mL;

(g) transferring the isolated cells from cord blood sample suspension to a 250 mL sterile bottle;

(h) adding additional CS10 to bring the volume of the isolated cells from cord blood sample suspension to about 157 mL;

(i) inserting a tube into the bottle comprising the isolated cells from cord blood sample suspension and priming the tubing until some isolated cells from cord blood sample suspension is dispensed into a reservoir;

(j) placing a 48-vial rack on a rack nest; and

(k) selecting a desired dispensing volume of 1000µL on the automated filling machine;

(l) operating the automated filling machine to fill the vials in the multi-vial rack; and

(m) transferring the filled cryovial to the controlled rate freezer (CRF).

15. The method of claim 13 or claim 14, further comprising

allowing a sample probe to reach about 4°C;

once at about 4°C, running the CRF;

upon completion of CRF, transferring the cryovials to an LN2 tank.

16. The method of claim 15, wherein transferring the cryovial to an LN2 tank, comprises first transferring the vials to a container with dry ice and then transferring the vial to an LN2 tank.

17. A cell suspension comprising:

isolated cells from cord blood sample; and

CS10 medium.

18. The cell suspension of claim 17, wherein the isolated cells from cord blood sample is isolated by any one of the methods of claims 1-10 and cryopreserved by any one of the methods of claim 13-16.

19. A method of processing and enrichment of cord blood unit (CBU) isolated cell fraction, comprising:

(a) cell fraction thawing and seeding, comprising:

adding StemSpan AOF into a vial containing frozen CBU isolated cell fraction;

thawing each vial;

transferring the thawed vial contents into a tube ("cells tube");

using Stem Span AOF to rinse the vial and collect residual cells;

transferring the suspension into the cells tube;

adding StemSpan AOF into the cells tube;

centrifuge the cells tube at 500xG-1000xG for 5-15 minutes resulting in supernatant and pellet;

discarding the supernatant;

resuspending the pellet with complete media;

optionally pooling the cell suspension in the cells tube ("first cells tube") with one or more additional cell suspension into one tube;

(b) cell fraction feeding 1, comprising:

transferring a calculated volume of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into a flask;

transferring a calculated volume of the cell suspension to the flask;

incubating the cell suspension in the flask;

(c) cell fraction feeding 2, comprising:

collecting the cell suspension in the flask and dispense it down the bottom of the flask 2-3 times;

transferring the suspension from the flask into a conical tube;

adding complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into the flask to rinse and collect residual cells;

transferring the suspension from the flask into the conical tube;

centrifuging the conical tube at 500-1000xG for 10 minutes at room temperature resulting in supernatant and pellet;

remove the supernatant, leaving behind approximately 5 mL;

resuspending the pellet with 30 mL of Stem Span Complete Media;

transferring the suspension into a flask;

incubating the cell suspension in the flask;

(d) cell fraction feeding 3, comprising:

collecting the cell suspension in the flask and dispense it down the bottom of the flask 2-3 times;

transferring the suspension from the flask into a conical tube;

adding 5 mL of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into the flask to rinse and collect residual cells;

transferring the suspension from the flask into the conical tube;

centrifuging the conical tube at 500xG-1000xG for 5-15 minutes resulting in supernatant and pellet;

remove the supernatant, leaving behind approximately 5 mL;

resuspending the pellet with Stem Span Complete Media;

transferring the suspension into a flask;

incubating the cell suspension in the flask.

20. A method of claim 19, wherein processing and enrichment of cord blood unit (CBU) isolated cell fraction, comprises:

(a) cell fraction thawing and seeding, comprising:

adding about 4.5 mL of StemSpan AOF into a vial containing frozen CBU isolated cell fraction;

thawing each vial in an about 37°C beaker in a bead bath;

transferring the thawed vial contents into a tube ("cells tube");

using about 4.5 mL of Stem Span AOF to rinse the vial and collect residual cells;

transferring the suspension into the cells tube;

adding about 31.5 mL of StemSpan AOF into the cells tube to yield a total volume of about 45mL;

centrifuge the cells tube at about 600xG for about 10 minutes at about room temperature resulting in supernatant and pellet;

discarding the supernatant;

resuspending the pellet with about 1 mL of complete media;

optionally pooling the cell suspension in the cells tube ("first cells tube") with one or more additional cell suspension into one tube;

(b) cell fraction feeding 1, comprising:

transferring a calculated volume of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into a flask;

transferring a calculated volume of the cell suspension to the flask;

incubating the cell suspension in the flask;

(c) cell fraction feeding 2, comprising:

collecting the cell suspension in the flask and dispense it down the bottom of the flask about 2-3 times;

transferring the suspension from the flask into a conical tube;

adding about 5 mL of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into the flask to rinse and collect residual cells;

transferring the suspension from the flask into the conical tube;

centrifuging the conical tube at about 600xG for about 10 minutes at room temperature resulting in supernatant and pellet;

remove the supernatant, leaving behind approximately about 5 mL;

resuspending the pellet with about 30 mL of complete media;

transferring the suspension into a flask;

incubating the cell suspension in the flask;

(d) cell fraction feeding 3, comprising:

collecting the cell suspension in the flask and dispense it down the bottom of the flask about 2-3 times;

transferring the suspension from the flask into a conical tube;

adding about 5 mL of complete media comprising StemSpan AOF and Stem Span CD34+ Expansion Supplement into the flask to rinse and collect residual cells;

transferring the suspension from the flask into the conical tube;

centrifuging the conical tube at about 600xG for about 10 minutes at room temperature resulting in supernatant and pellet;

remove the supernatant, leaving behind approximately about 5 mL;

resuspending the pellet with about 30 mL of complete media;

transferring the suspension into a flask;

incubating the cell suspension in the flask.

21. The method of any one of claims 19 or 20, further comprising cell fraction harvesting on day of reprogramming.

22. The method of any one of claims 19 or 20, wherein the complete media comprising StemSpan AOF and StemSpan CD34+ Expansion Supplement are an about 9:1 ratio of StemSpan AOF:StemSpan CD34+ Expansion Supplement.

23. The method of any one of claims 19 or 20, wherein optionally pooling the cell suspension in the cells tube ("first cells tube") with one or more additional cell suspension into one tube, comprises

transferring the cell suspension from the one or more additional cells tubes to the first cells tube;

adding about 1 mL of complete media into the one or more additional cells tubes to wash and collect residual cells, and transferring the suspension from the one or more additional cells tubes to the first cells tube.

24. A method of washing a frozen cord blood sample, isolating cells, and processing and enrichment of cord blood unit (CBU) isolated cell fraction, comprising

performing the method steps of any one of claims 1-16; and

performing the method steps of any one of claims 19-23.

25. A quantity of hematopoietic stem/progenitor cells isolated from cord blood, wherein the hematopoietic stem/progenitor cells comprise cells selected from erythroid progenitors, monocyte and macrophages, granulocyte progenitors and lymphoid cells or combinations thereof, and wherein the hematopoietic stem/progenitor cells express one or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22.

26. The quantity of isolated cells of claim 25, wherein the hematopoietic stem/progenitor cells express one or more hematopoietic stem/ progenitor marker genes selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22.

27. A cell suspension comprising:

isolated cell fraction (ICF) from Cord Blood Units (CBUs); and

complete media, wherein complete media comprises Stem-Span AOF and StemSpan CD34+ Expansion Supplement.

28. The cell suspension of claim 27, wherein the ICF comprises hematopoietic stem/progenitor cells selected from erythroid progenitors, monocyte and macrophages, granulocyte progenitors and lymphoid cells or combinations thereof, and wherein the hematopoietic stem/progenitor cells express one or more hematopoietic stem/ progenitor marker genes

selected from CD11a (ITGAL), CD11c (ITGAX), CD34, CD49d (ITGA4), RUNX1, GATA2, TAL1, CBFAT2T3, MMLT3, or PTPN22.