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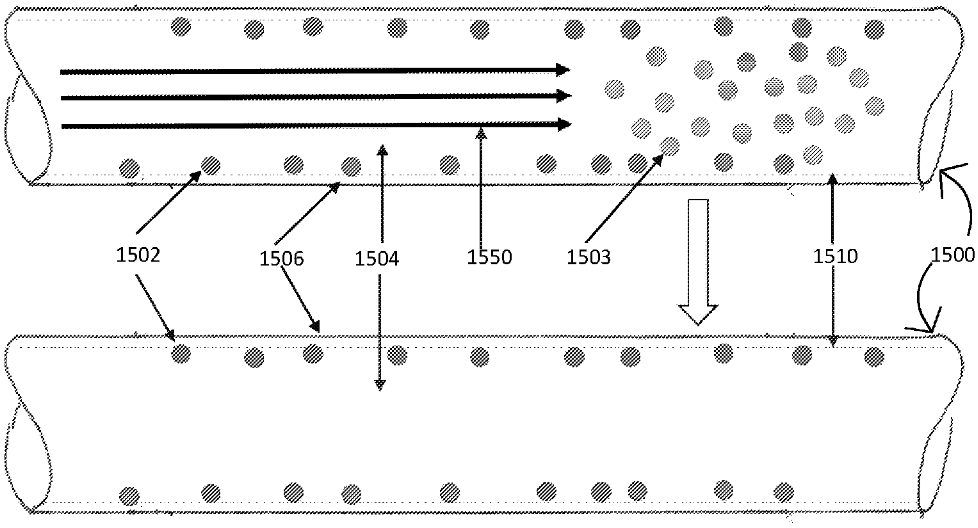

FIG. 16

(57) **Abstract:** Implementations are described that relate to methods and systems for growing cells in a hollow fiber bioreactor. In implementations, the cells may be exposed to a number of growth factors including a combination of recombinant growth factors. In other implementations, the cells may be grown in co-culture with other cells, e.g., hMSC's. In implementations, the cells may include CD34+ cells. A coated membrane includes a membrane having a first coating configured to promote cellular adhesion to the membrane and a second coating that includes a soluble protein moiety.

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CELL CAPTURE AND EXPANSION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of and priority, under 35 U.S.C. § 119(e), to the following U.S. Provisional Patent Application Serial Nos.: 63/165,060, filed on March 23, 2021, entitled “Cell Expansion”; 63/169,173, filed on March 31, 2021, entitled “Cell Expansion”; 63/183,591, filed on May 3, 2021, entitled “ Cell Expansion”; 63/227,293, filed on July 29, 2021, entitled “Cell Expansion”; 63/228,561, filed on August 2, 2021, entitled “Cell Expansion”; 63/275,389, filed on November 3, 2021, entitled “Methods and Systems for Isolating Target Cells Using a Multiple Part Membrane Substrate”; 63/275,793, filed on November 4, 2021, entitled “Methods and Systems for Isolating Target Cells Using a Multiple Part Membrane Substrate”; 63/304,467, filed on January 28, 2022, entitled “Methods and Systems for Isolating Target Cells Using a Multiple Part Membrane Substrate”; the entire disclosures of each are incorporated herein by reference, in their entirety.

BACKGROUND

[0002] The present disclosure is generally directed to isolating and expanding living cells, in particular, toward isolating target cells using a membrane and expanding the isolated cells.

[0003] Cell processing systems include Cell Collection Systems and Cell Expansion Systems (CES’s). Cell Collection Systems collect cells from a supply source and CES’s may be used to expand and differentiate a variety of cell types. Expanded and/or differentiated cells may be used for both research and/or therapeutic purposes. As one example, hematopoietic stem cells (HSC) possess multipotentiality, enabling them to self-renew and to produce mature blood cells, such as erythrocytes, leukocytes, platelets, and lymphocytes. CD34 is a marker of human HSC, and all colony-forming activity of human bone marrow (BM) cells is found in the fraction of cells expressing CD34 (i.e., “CD34+ HSCs” or “CD34+ cells” or “the CD34+ fraction”). HSC’s may be collected from bone marrow, cord blood, or peripheral blood, and CD34+ HSCs have been identified as possible treatments for diseases such as hematological cancers (e.g., lymphoma, leukemia, myeloma). Umbilical cord blood (CB) is increasingly being used as an alternative to bone marrow (BM) as a source of transplantable CD34+ HSCs. Effective treatment with, or transplantation of, CD34+ HSCs requires the administration of a minimum number of

HSC's. Accordingly, following isolation of CD34+ HSCs from a suitable source, such as CB, the CD34+ HSCs must be grown (i.e., "expanded") from an initial amount to at least an amount that may be considered effective for treatment or transplantation.

[0004] This disclosure provides procedures, devices, and compositions useful in the isolation, expansion, and administration of CD34+ HSCs.

SUMMARY

[0005] This summary is provided to introduce aspects of this disclosure in a simplified form, and is not intended to identify key or essential elements, nor is it intended to limit the scope of the claims.

[0006] This disclosure provides cell capture and expansion systems and methods of expanding target cells that may be collected from a mixed cell population. Examples include a membrane useful for trapping, collecting, and/or otherwise holding target cells, in particular CD34+ HSCs. Using the methods of this disclosure, the HSCs may be collected and significantly expanded quickly and efficiently while minimizing or eliminating differentiation of the HSCs. In the systems and methods of this disclosure, the HSCs may be expanded at least 50-fold. The cells may be target cells collected from a donor fluid (e.g., one or more blood components). These target cells may include, but are not limited to, stem cells, CD34+ HSCs, T-cells, natural killer (NK) cells, monocytes, or the like. The membrane may comprise one or more layers or coatings (i.e., a membrane) that are configured to attract and collect target cells. The membrane may comprise a substrate that promotes cellular adhesion to at least one surface of the substrate. The substrate may have a first surface and a second surface and at least one coating on the first surface and/or the second surface. The at least one coating may correspond to any molecule or material that promotes cellular adhesion to the first surface and/or the second surface of the substrate. The at least one coating may include a first coating material and a second coating material. The first coating material may be fibronectin, or a fibronectin equivalent, and the second coating material may be a soluble protein moiety. The second coating material may target specific target cells from a mixed cell population. For instance, the second coating material may be a chemokine, such as stromal cell-derived factor-1 (SDF-1), which may be used to enhance collection of CD34+ HSCs. Additional coating materials may be used to collect the same or different cells from a mixed cell population. The membrane may be arranged in any form, such as a flat sheet, a filter matrix, a hollow fiber, any combination thereof, and/or any plurality thereof.

[0007] This disclosure also provides methods for expanding cells, in particular CD34+ HSCs, in a bioreactor, such as a hollow fiber bioreactor. These methods provide for introducing cells (e.g., hematopoietic stem cells (HSC's), including, for example, CD34+ HSCs) into a bioreactor, and exposing the cells to growth conditions that expand the number of cells in the bioreactor. The growth conditions may include the introduction of one or a combination of growth factors into bioreactor. Alternatively or additionally, the growth conditions may include the presence of co-cultured cells in the bioreactor. After expanding the cells in the bioreactor, a plurality of expanded cells may then be removed from the bioreactor for storage, transplantation, or use in therapies such as cancer therapies.

[0008] This disclosure provides methods of expanding cells that include introducing a plurality of cells comprising CD34+ Hematopoietic stem cells (HSCs) into hollow fibers of a hollow fiber bioreactor. The hollow fibers of the bioreactor each comprise an interior lumen and an extracapillary side. Additionally, the hollow fibers comprise a coating on at least one of the lumen surface and the extracapillary surface. The coating on the surface(s) includes stromal cell-derived factor-1 (SDF-1) and fibronectin or isoforms, or functional equivalents thereof. In these methods, the plurality of cells in the hollow fibers are exposed to growth conditions and at least a portion of the plurality of cells is expanded in the hollow fibers of the bioreactor to generate a plurality of expanded CD34+ HSCs. Using these methods, the plurality of cells introduced into the hollow fibers of the bioreactor may be expanded at least 50-fold.

[0009] This disclosure also provides methods of expanding cells by perfusion in a cell expansion system. These methods include coating a hollow fiber bioreactor with a first fluid, which may include a signaling factor and/or a coating factor. In these methods, a plurality of cells is introduced into a hollow fiber membrane of a hollow fiber bioreactor. In these methods, the plurality of cells in the hollow fiber membrane may be exposed to a second fluid, which includes a plurality of growth factors. In these methods, the plurality of cells in the hollow fiber bioreactor may be grown in monoculture or in coculture.

[0010] This disclosure also provides methods of capturing cells that includes introducing a mixture of target cells and non-target cells into hollow fibers of a hollow fiber bioreactor. These hollow fibers each comprise an interior lumen and an extracapillary side, and a coating on at least one of the lumen surface and the extracapillary surface of the hollow fibers. The coating on the surface(s) includes stromal cell-derived factor-1 (SDF-1) and fibronectin or isoforms, or functional equivalents thereof. In these methods, the mixture of target and non-target cells in the hollow fibers may be exposed to capture conditions to capture at least a portion of the target cells on at least one of the lumen and the extracapillary surface of the hollow fibers. At least a portion of the non-target cells may be flushed from the hollow fibers, leaving target cells associated with a surface of the hollow fibers.

[0011] This disclosure also provides methods of capturing target species. In these methods, a mixture of target species and non-target species are introduced into hollow fibers, which have an interior lumen and an extracapillary side. These hollow fibers may include a coating on at least one of the lumen surface and the extracapillary surface of the hollow fibers. The coating may include at least one of streptavidin, avidin, a biotinylated molecule, and an anti-biotin antibody or a functional fragment thereof. In these methods, the mixture of target species and non-target species in the hollow fibers may be exposed to capture conditions to capture at least a portion of the target species on at least one of the lumen and the extracapillary surface(s) of the hollow fibers. In these methods, at least a portion of the non-target species may be flushed from the hollow fibers.

[0012] This disclosure also provides coated hollow fiber membranes. These membranes are hollow fiber membranes having a lumen surface and an extracapillary surface. These membranes may include a coating on at least one of the lumen surface and the extracapillary surface of the hollow fibers. The coating may include stromal cell-derived factor-1 (SDF-1) and fibronectin or isoforms, or functional equivalents thereof.

[0013] This disclosure also provides methods of forming a coated hollow fiber membrane that include providing a hollow fiber membrane having a lumen surface and an extracapillary surface and applying a first coating onto the lumen surface of the hollow fiber membrane. In these methods, the first coating comprises a material that promotes cellular adhesion to at least one of the lumen of the hollow fiber membrane and the extracapillary surface of the hollow fiber membrane. In these methods, a second coating may be applied onto the lumen surface of the hollow fiber membrane. The second coating may include a soluble protein moiety.

[0014] This disclosure also provides compositions useful for expanding CD34+ HSCs. These compositions include glial cell-derived neurotrophic factor (GDNF) and an aryl hydrocarbon receptor (AHR) antagonist.

[0015] The preceding is intended to provide a simplified summary of some aspects of the disclosure. This summary is neither an extensive nor exhaustive overview of the disclosure and its various aspects, implementations, and configurations. It is intended neither to identify key or critical elements of the disclosure nor to delineate the scope of the disclosure but to present selected concepts of the disclosure in a simplified form as an introduction to the more detailed description presented below. As will be appreciated, other aspects, implementations, and configurations of the disclosure are possible utilizing, alone or in combination, one or more of the features set forth above or described in detail below, and will be apparent to those skilled in the art upon consideration of the following Detailed Description and in view of the Figures.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0016] The accompanying drawings are incorporated into and form a part of the specification to illustrate several examples of the present disclosure. These drawings, together with the description, explain the principles of the disclosure. The drawings simply illustrate preferred and alternative examples of how the disclosure can be made and used and are not to be construed as limiting the disclosure to only the illustrated and described examples.

[0017] FIG. 1 depicts a perspective view of a hollow fiber bioreactor, in accordance with implementations.

[0018] FIG. 2 illustrates a perspective view of a cell expansion system with a premounted fluid conveyance device, in accordance with implementations.

[0019] FIG. 3 depicts a perspective view of a housing of a cell expansion system, in accordance with implementations.

[0020] FIG. 4 illustrates a perspective view of a premounted fluid conveyance device, in accordance with implementations.

[0021] FIG. 5 depicts a schematic of a cell expansion system, in accordance with implementations.

[0022] FIG. 6 illustrates a schematic of another implementation of a cell expansion system, in accordance with implementations.

[0023] FIG. 7 illustrates components of a computing system that may be used to implement implementations.

[0024] FIG. 8 shows a schematic representation of a hollow fiber in accordance with implementations of the present disclosure.

[0025] FIG. 9 shows a schematic representation of ultrafiltration from the lumen side to the extracapillary side of the hollow fiber in accordance with an implementations of the present disclosure.

[0026] FIG. 10 shows a schematic representation of flow stopped for segregating mixed cell populations in accordance with implementations of the present disclosure.

[0027] FIG. 11 is a schematic representation showing cells falling to the bottom of the hollow fiber in accordance with implementations of the present disclosure.

[0028] FIG. 12 is a schematic representation showing a membrane coated with a mixture of fibronectin and SDF-1.

[0029] FIG. 13 is a schematic representation showing a cell suspension in a coated membrane.

[0030] FIG. 14 illustrates flow 1400 that may be performed in embodiments to expand cells (e.g., HSCs). Although specific devices may be described below for performing steps in flow 1400, embodiments are not limited thereto. For example, some steps may be described as performed by parts of a cell expansion system (e.g., CES's 500 or 600) or a processor (1100 (FIG. 7)), which may execute steps based on software provided as processor executable instructions. This is done merely for illustrative purposes, and flow 1400 is not limited to being performed by any specific device.

[0031] FIG. 15 is a schematic representation depicting a suspension of target and non-target species in the lumen of a hollow fiber having a coating on the lumen surface of the hollow fiber.

[0032] FIG. 16 is a schematic representation depicting the capture of target cells on the coating material on the lumen of a hollow fiber.

[0033] FIG. 17 is a graph showing $CD34^+$ cell harvest yield from three different donor cell lineages after 8 days of monoculture compared to the minimum single and double CBU CD34+ cell dosing guidelines for a 70 kg patient.

[0034] FIG. 18 is a graph showing harvest cell viability as determined by trypan blue dye exclusion.

[0035] FIG. 19 is a graph showing the correlation of pre-cryopreservation cell viability with cord blood-derived $CD34^+$ cell harvest yield, with Pearson’s correlation coefficient of $R^2 = 0.8863$.

[0036] FIG. 20 is a graph showing the mean of CD34+ normalized glucose consumption rate (mmol/day) and lactate generation rate (mmol/day).

[0037] FIG. 21A and FIG. 21B show the FMO gating strategy (FSC-A vs SSC-A → singlets FSC-H vs FSC-A → live cells SSC-A vs &-AAD-A → SSC-A vs CD45-APC-H7 → SSC-A (FIG. 21A) vs CD34-APC → CD133-PE vs CD38-BB515 (FIG. 21B)) may be verified with Streck CD-Chex-CD34 Level 3 peripheral blood reference standard. 10,000 events were acquired per sample.

[0038] FIG. 22 is a graph showing differentiated colony forming units (CFUs) from Quantum-expanded CD34+ HSCs 14 days post-harvest, with 6 replicates per donor cell line.

[0039] FIG. 23 shows representative images of CFU-granulocyte, erythroid, macrophage, megakaryocyte, CFU-granulocyte and macrophage, and BFU-erythroid lineages.

[0040] FIG. 24 illustrates flow 2400 that may be performed in embodiments to capture cells (e.g., HSCs). Although specific devices may be described below for performing steps in flow 2400, embodiments are not limited thereto. For example, some steps may be described as performed by parts of a cell processing system (e.g., CES's 500 or 600) or a processor (1100 (FIG. 7)), which may execute steps based on software provided as processor executable instructions. This is done merely for illustrative purposes, and flow 2400 is not limited to being performed by any specific device.

DETAILED DESCRIPTION

[0041] The principles of the present disclosure may be further understood by reference to the following detailed description and the implementations depicted in the accompanying drawings. It should be understood that although specific features are shown and described below with respect to detailed implementations, the present disclosure is not limited to the implementations described below.

[0042] Reference will now be made in detail to the implementations illustrated in the accompanying drawings and described below. Wherever possible, the same reference numerals are used in the drawings and the description to refer to the same or like parts.

[0043] Referring to FIG. 1, an example of a hollow fiber bioreactor 100, which may be used with the present disclosure is shown in front side elevation view. Hollow fiber bioreactor 100 has a longitudinal axis LA-LA and includes chamber housing 104. In at least one implementation, chamber housing 104 includes four openings or ports: intracapillary (IC) inlet port 108, IC outlet port 120, extracapillary (EC) inlet port 128, and EC outlet port 132.

[0044] According to implementations of the present disclosure, fluid in a first circulation path enters hollow fiber bioreactor 100 through IC inlet port 108 at a first longitudinal end

112 of the hollow fiber bioreactor 100, passes into and through the intracapillary side (referred to in various implementations as the lumen, intracapillary (“IC”) side, or “IC space” of a hollow fiber membrane) of a plurality of hollow fibers 116, and out of hollow fiber bioreactor 100 through IC outlet port 120 located at a second longitudinal end 124 of the hollow fiber bioreactor 100. The fluid path between the IC inlet port 108 and the IC outlet port 120 defines the IC portion 126 of the hollow fiber bioreactor 100. Fluid in a second circulation path flows in the hollow fiber bioreactor 100 through EC inlet port 128, comes in contact with the extracapillary side or outside (referred to as the “EC side” or “EC space” of the membrane) of the hollow fibers 116, and exits hollow fiber bioreactor 100 via EC outlet port 132. The fluid path between the EC inlet port 128 and the EC outlet port 132 comprises the EC portion 136 of the hollow fiber bioreactor 100. Fluid entering hollow fiber bioreactor 100 via the EC inlet port 128 may be in contact with the outside of the hollow fibers 116. Small molecules (e.g., ions, water, oxygen, lactate) may diffuse through the hollow fibers 116 from the interior or IC space of the hollow fiber to the exterior or EC space, or from the EC space to the IC space. Large molecular weight molecules, such as growth factors, may be too large to pass through the hollow fiber membrane, and remain in the IC space of the hollow fibers 116. The media may be replaced as needed, in implementations. Media may also be circulated through an oxygenator or gas transfer module to exchange gasses as needed (see e.g., cell expansion systems 500 (FIG. 5) and 600 (FIG. 6)). Cells may be contained within a first circulation path and/or a second circulation path, as described below, and may be on either the IC side and/or EC side of the membrane, according to implementations.

[0045] The material used to make the hollow fiber membrane may be any biocompatible polymeric material which is capable of being made into hollow fibers and which possesses suitable permeability to small molecules such as, for example, ions, water, oxygen, glucose and lactate. One material which may be used is a synthetic polysulfone-based material, according to an implementation of the present disclosure. For the cells to adhere to the surface of the hollow fibers, the surface may be modified in some way, either by coating at least the cell growth surface with a protein, e.g., a glycoprotein such as fibronectin or collagen, or by exposing the surface to radiation. Gamma treating the membrane surface may allow for attachment of adherent cells without additionally coating the membrane with fibronectin or the like. Other coatings and/or treatments for cell attachment may be used in accordance with implementations of the present disclosure.

[0046] Turning to FIG. 2, an implementation of a cell expansion system 200 with a premounted fluid conveyance assembly is shown in accordance with implementations of the present disclosure. The CES 200 includes a cell expansion machine 202 that comprises a hatch or closable door 204 for engagement with a back portion 206 of the cell expansion machine 202. An interior space 208 within the cell expansion machine 202 includes features adapted for receiving and engaging a premounted fluid conveyance assembly 210 that includes a bioreactor 100. The premounted fluid conveyance assembly 210 may be detachably-attachable to the cell expansion machine 202 to facilitate relatively quick exchange of a new or unused premounted fluid conveyance assembly 210 at a cell expansion machine 202 for a used premounted fluid conveyance assembly 210 at the same cell expansion machine 202. A single cell expansion machine 202 may be operated to grow or expand a first set of cells using a first premounted fluid conveyance assembly 210 and, thereafter, may be used to grow or expand a second set of cells using a second premounted fluid conveyance assembly 210 without needing to be sanitized between interchanging the first premounted fluid conveyance assembly 210 for the second premounted fluid conveyance assembly 210. The premounted fluid conveyance assembly includes a bioreactor 100 and an oxygenator or gas transfer module 212. Tubing guide slots are shown as 214 for receiving various media tubing connected to premounted fluid conveyance assembly 210, according to implementations.

[0047] Next, FIG. 3 illustrates the back portion 206 of cell expansion machine 202 prior to detachably-attaching a premounted fluid conveyance assembly 210 (FIG. 2), in accordance with implementations of the present disclosure. The closable door 204 (shown in FIG. 2) is omitted from FIG. 3. The back portion 206 of the cell expansion machine 202 includes a number of different structures for working in combination with elements of a premounted fluid conveyance assembly 210. More particularly, the back portion 206 of the cell expansion machine 202 includes a plurality of peristaltic pumps for cooperating with pump loops on the premounted fluid conveyance assembly 210, including the IC circulation pump 218, the EC circulation pump 220, the IC inlet pump 222, and the EC inlet pump 224. In addition, the back portion 206 of the cell expansion machine 202 includes a plurality of valves, including the IC circulation valve 226, the reagent valve 228, the IC media valve 230, the air removal valve 232, the cell inlet valve 234, the wash valve 236, the distribution valve 238, the EC media valve 240, the IC waste valve 242, the EC waste valve 244, and the harvest valve 246. Several sensors are also associated with the back portion 206 of the cell expansion machine 202, including the IC outlet pressure sensor 248, the combination IC inlet pressure and temperature sensors 250, the combination EC inlet pressure and temperature sensors 252, and the EC outlet pressure sensor 254. Also shown is an optical sensor 256 for an air removal chamber.

[0048] In accordance with implementations, a shaft or rocker control 258 for rotating the bioreactor 100 is shown in FIG. 3. Shaft fitting 260 associated with the shaft or rocker control 258 allows for proper alignment of a shaft access aperture, see e.g., 424 (FIG. 4) of a tubing-organizer, see e.g., 300 (FIG. 4) of a premounted conveyance assembly 210 or 400 with the back portion 206 of the cell expansion machine 202. Rotation of shaft or rocker control 258 imparts rotational movement to shaft fitting 260 and bioreactor 100. Thus, when an operator or user of the CES 200 attaches a new or unused premounted fluid conveyance assembly 400 (FIG. 4) to the cell expansion machine 202, the alignment is a relatively simple matter of properly orienting the shaft access aperture 424 (FIG. 4) of the premounted fluid conveyance assembly 400 with the shaft fitting 260.

[0049] Turning to FIG. 4, a perspective view of a detachably-attachable premounted fluid conveyance assembly 400 is shown. The premounted fluid conveyance assembly 400 may be detachably-attachable to the cell expansion machine 202 to facilitate relatively quick exchange of a new or unused premounted fluid conveyance assembly 400 at a cell expansion machine 202 for a used premounted fluid conveyance assembly 400 at the same cell expansion machine 202. As shown in FIG. 4, the bioreactor 100 may be attached to a bioreactor coupling that includes a shaft fitting 402. The shaft fitting 402 includes one or more shaft fastening mechanisms, such as a biased arm or spring member 404 for engaging a shaft, e.g., 258 (shown in FIG. 3), of the cell expansion machine 202.

[0050] In implementations, the shaft fitting 402 and the spring member 404 connect to mechanisms of a cell expansion system that rotate the bioreactor 100. For example, in some implementations, the cell expansion system may be part of a QUANTUM® Cell Expansion System (CES), manufactured by Terumo BCT, Inc. of Lakewood, CO, which provides for rotation of a bioreactor. Examples of cell expansion systems that provide for rotation of the bioreactor are described in at least: U.S. Patent No. 8,399,245, issued March 19, 2013, entitled "ROTATION SYSTEM FOR CELL GROWTH CHAMBER OF A CELL EXPANSION SYSTEM AND METHOD OF USE THEREFOR;" U.S. Patent No. 8,809,043, issued February 13, 2013, entitled "ROTATION SYSTEM FOR CELL GROWTH CHAMBER OF A CELL EXPANSION SYSTEM AND METHOD OF USE THEREFOR;" and U.S. Patent No. 9,057,045, issued June 16, 2015, entitled "METHOD OF LOADING AND DISTRIBUTING CELLS IN A BIOREACTOR OF A CELL

EXPANSION SYSTEM;" all three of which are hereby incorporated by reference in their entirety as if set forth herein in full.

[0051] According to implementations, the premounted fluid conveyance assembly 400 includes tubing 408A, 408B, 408C, 408D, 408E, and various tubing fittings to provide the fluid paths shown in FIGS. 5 and 6, as discussed below. Pump loops 406A and 406B are also provided for the pump(s). In implementations, although the various media may be provided at the site where the cell expansion machine 202 is located, the premounted fluid conveyance assembly 400 may include sufficient tubing length to extend to the exterior of the cell expansion machine 202 and to enable welded connections to tubing associated with the media bags, according to implementations.

[0052] FIG. 5 illustrates a schematic of an implementation of a cell expansion system 500, and FIG. 6 illustrates a schematic of another implementation of a cell expansion system 600. In the implementations shown in FIGS. 5 and 6, and as described below, the cells are grown in the IC space and may in other implementations provide for cells to be grown in the EC space. In yet other implementations, such as when co-culturing cells, first cells may be grown in the EC space, while second cells may be grown in the IC space. Co-culturing of cells may also be performed by growing first cells and second cells in the EC space, or growing first cells and second cells in the IC space.

[0053] FIG. 5 illustrates a CES 500, which includes first fluid circulation path 502 (also referred to as the "intracapillary loop" or "IC loop") and second fluid circulation path 504 (also referred to as the "extracapillary loop" or "EC loop"), according to implementations. First fluid flow path 506 may be fluidly associated with hollow fiber bioreactor 501 to form, at least in part, first fluid circulation path 502. Fluid flows into hollow fiber bioreactor 501 through IC inlet port 501A, through hollow fibers in hollow fiber bioreactor 501, and exits via IC outlet port 501B. Pressure gauge 510 measures the pressure of media leaving hollow fiber bioreactor 501. Media flows through IC circulation pump 512 which may be used to control the rate of media flow/rate of fluid circulation. IC circulation pump 512 may pump the fluid in a first direction (e.g., clockwise) or second direction opposite the first direction (e.g., counter clockwise). Exit port 501B may be used as an inlet in the reverse direction. Media entering the IC loop 502 may then enter through valve 514. As those skilled in the art will appreciate, additional valves and/or other devices may be placed at various locations to isolate and/or measure characteristics of the media along portions of the fluid paths. Accordingly, it is to be understood that the schematic shown represents one possible configuration for various elements of the CES

500, and modifications to the schematic shown are within the scope of the one or more present implementations.

[0054] With regard to the IC loop 502, samples of media may be obtained from sample port 516 or sample coil 518 during operation. Pressure/temperature gauge 520 disposed in first fluid circulation path 502 allows detection of media pressure and temperature during operation. Media then returns to IC inlet port 501A to complete fluid circulation path 502. Cells grown/expanded in hollow fiber bioreactor 501 may be flushed out of hollow fiber bioreactor 501 into harvest bag 599 through valve 598 or redistributed within the hollow fibers for further growth.

[0055] Fluid in second fluid circulation path 504 enters hollow fiber bioreactor 501 via EC inlet port 501C, and leaves hollow fiber bioreactor 501 via EC outlet port 501D. Media in the EC loop 504 may be in contact with the outside of the hollow fibers in the hollow fiber bioreactor 501, thereby allowing diffusion of small molecules into and out of the hollow fibers.

[0056] Pressure/temperature gauge 524 disposed in the second fluid circulation path 504 allows the pressure and temperature of media to be measured before the media enters the EC space of hollow fiber bioreactor 501. Pressure gauge 526 allows the pressure of media in the second fluid circulation path 504 to be measured after it leaves hollow fiber bioreactor 501. With regard to the EC loop, samples of media may be obtained from sample port 530 or a sample coil during operation.

[0057] In implementations, after leaving EC outlet port 501D of hollow fiber bioreactor 501, fluid in second fluid circulation path 504 passes through EC circulation pump 528 to oxygenator or gas transfer module 532. EC circulation pump 528 may also pump the fluid in opposing directions. Second fluid flow path 522 may be fluidly associated with oxygenator or gas transfer module 532 via oxygenator inlet port 534 and oxygenator outlet port 536. In operation, fluid media flows into oxygenator or gas transfer module 532 via oxygenator inlet port 534, and exits oxygenator or gas transfer module 532 via oxygenator outlet port 536. Oxygenator or gas transfer module 532 adds oxygen to and removes both carbon dioxide and bubbles from media in the CES 500. In various implementations, media in second fluid circulation path 504 may be in equilibrium with gas entering oxygenator or gas transfer module 532. The oxygenator or gas transfer module 532 may be any appropriately sized oxygenator or gas transfer device. Air or gas flows into oxygenator or gas transfer module 532 via filter 538 and out of oxygenator or gas transfer device 532 through filter 540. Filters 538 and 540 reduce or prevent contamination of oxygenator or gas transfer module 532 and associated media. Air or gas purged from the CES 500 during portions of a priming sequence may vent to the atmosphere via the oxygenator or gas transfer module 532.

[0058] In the configuration depicted for CES 500, fluid media in first fluid circulation path 502 and second fluid circulation path 504 flows through hollow fiber bioreactor 501 in the same direction (a co-current configuration). The CES 500 may also be configured to flow in a counter-current configuration.

[0059] In accordance with at least one implementation, media, including cells (from bag 562), and fluid media from bag 546 may be introduced to first fluid circulation path 502 via first fluid flow path 506. Fluid container 562 (e.g., Cell Inlet Bag or Saline Priming Fluid for priming air out of the system) may be fluidly associated with the first fluid flow path 506 and the first fluid circulation path 502 via valve 564.

[0060] Fluid containers, or media bags, 544 (e.g., Reagent) and 546 (e.g., IC Media) may be fluidly associated with either first fluid inlet path 542 via valves 548 and 550, respectively, or second fluid inlet path 574 via valves 548, 550, and 570. First and second sterile sealable input priming paths 508 and 509 are also provided. An air removal chamber (ARC) 556 may be fluidly associated with first circulation path 502. The air removal chamber 556 may include one or more ultrasonic sensors including an upper sensor and lower sensor to detect air, a lack of fluid, and/or a gas/fluid interface, e.g., an air/fluid interface, at certain measuring positions within the air removal chamber 556. For example, ultrasonic sensors may be used near the bottom and/or near the top of the air removal chamber 556 to detect air, fluid, and/or an air/fluid interface at these locations. Implementations provide for the use of numerous other types of sensors without departing from the spirit and scope of the present disclosure. For example, optical sensors may be used in accordance with implementations of the present disclosure. Air or gas purged from the CES 500 during portions of the priming sequence or other protocols may vent to the atmosphere out air valve 560 via line 558 that may be fluidly associated with air removal chamber 556.

[0061] EC media (from bag 568) or wash solution (from bag 566) may be added to either the first or second fluid flow paths. Fluid container 566 may be fluidly associated with valve 570 that may be fluidly associated with first fluid circulation path 502 via distribution valve 572 and first fluid inlet path 542. Alternatively, fluid container 566 may be fluidly associated with second fluid circulation path 504 via second fluid inlet path 574 and EC inlet path 584 by opening valve 570 and closing distribution valve 572. Likewise, fluid container 568 may be fluidly associated with valve 576 that may be fluidly associated with first fluid circulation path 502 via first fluid inlet path 542 and distribution valve 572. Alternatively, fluid container 568 may be fluidly associated with second fluid inlet path 574 by opening valve 576 and closing valve distribution 572. An optional heat exchanger 552 may be provided for media reagent or wash solution introduction.

[0062] In the IC loop, fluid may be initially advanced by the IC inlet pump 554. In the EC loop, fluid may be initially advanced by the EC inlet pump 578. An air detector 580, such as an ultrasonic sensor, may also be associated with the EC inlet path 584.

[0063] In at least one implementation, first and second fluid circulation paths 502 and 504 are connected to waste line 588. When valve 590 is opened, IC media may flow through waste line 588 and to waste or outlet bag 586. Likewise, when valve 582 is opened, EC media may flow through waste line 588 to waste or outlet bag 586.

[0064] In implementations, cells may be harvested via cell harvest path 596. Here, cells from hollow fiber bioreactor 501 may be harvested by pumping the IC media containing the cells through cell harvest path 596 and valve 598 to cell harvest bag 599.

[0065] Various components of the CES 500 may be contained or housed within a machine or housing, such as cell expansion machine 202 (FIGS. 2 and 3), wherein the machine maintains cells and media at a predetermined temperature.

[0066] Turning to FIG. 6, a schematic of another implementation of a cell expansion system 600 is shown. CES 600 includes a first fluid circulation path 602 (also referred to as the “intracapillary loop” or “IC loop”) and second fluid circulation path 604 (also referred to as the “extracapillary loop” or “EC loop”). First fluid flow path 606 may be fluidly associated with hollow fiber bioreactor 601 to form first fluid circulation path 602. Fluid flows into hollow fiber bioreactor 601 through IC inlet port 601A, through hollow fibers in hollow fiber bioreactor 601, and exits via IC outlet port 601B. Pressure sensor 610 measures the pressure of media leaving hollow fiber bioreactor 601. In addition to pressure, sensor 610 may, in implementations, also be a temperature sensor that detects the media pressure and temperature during operation.

[0067] Media flows through IC circulation pump 612 which may be used to control the rate of media flow or rate of circulation. IC circulation pump 612 may pump the fluid in a first direction (e.g. counter clockwise) or second direction opposite the first direction (e.g., clockwise). Exit port 601B may be used as an inlet in the reverse direction. Media entering the IC loop may flow through valve 614. As those skilled in the art will appreciate, additional valves and/or other devices may be placed at various locations to isolate and/or measure characteristics of the media along portions of the fluid paths. Samples of media may be obtained from sample coil 618 during operation. Media then returns to IC inlet port 601A to complete fluid circulation path 602.

[0068] Cells grown/expanded in hollow fiber bioreactor 601 may be flushed out of hollow fiber bioreactor 601 into harvest bag 699 through valve 698 and line 697. Alternatively, when valve 698 is closed, the cells may be redistributed within hollow fiber bioreactor 601 for further growth. It is to be understood that the schematic shown represents one possible configuration for various elements of the CES 600, and modifications to the schematic shown are within the scope of the one or more present implementations.

[0069] Fluid in second fluid circulation path 604 enters hollow fiber bioreactor 601 via EC inlet port 601C and leaves hollow fiber bioreactor 601 via EC outlet port 601D. Media in the EC loop may be in contact with the outside of the hollow fibers in the hollow fiber bioreactor 601, thereby allowing diffusion of small molecules into and out of the hollow fibers that may be within chamber 601, according to an implementation.

[0070] Pressure/temperature sensor 624 disposed in the second fluid circulation path 604 allows the pressure and temperature of media to be measured before the media enters the EC space of the hollow fiber bioreactor 601. Sensor 626 allows the pressure and/or temperature of media in the second fluid circulation path 604 to be measured after it leaves the hollow fiber bioreactor 601. With regard to the EC loop, samples of media may be obtained from sample port 630 or a sample coil during operation.

[0071] After leaving EC outlet port 601D of hollow fiber bioreactor 601, fluid in second fluid circulation path 604 passes through EC circulation pump 628 to oxygenator or gas transfer module 632. EC circulation pump 628 may also pump the fluid in opposing directions, according to implementations. Second fluid flow path 622 may be fluidly associated with oxygenator or gas transfer module 632 via an inlet port 632A and an outlet port 632B of oxygenator or gas transfer module 632. In operation, fluid media flows into oxygenator or gas transfer module 632 via inlet port 632A, and exits oxygenator or gas transfer module 632 via outlet port 632B. Oxygenator or gas transfer module 632 adds oxygen to and removes both carbon dioxide and bubbles from media in the CES 600.

[0072] In various implementations, media in second fluid circulation path 604 may be in equilibrium with gas entering oxygenator or gas transfer module 632. The oxygenator or gas transfer module 632 may be any appropriately sized device useful for oxygenation or gas transfer. Air or gas flows into oxygenator or gas transfer module 632 via filter 638 and out of oxygenator or gas transfer device 632 through filter 640. Filters 638 and 640 reduce or prevent contamination of oxygenator or gas transfer module 632 and associated media. Air or gas purged from the CES 600 during portions of a priming sequence may vent to the atmosphere via the oxygenator or gas transfer module 632.

[0073] In the configuration depicted for CES 600, fluid media in first fluid circulation path 602 and second fluid circulation path 604 flows through hollow fiber bioreactor 601 in the same direction (a co-current configuration). The CES 600 may also be configured to flow in a counter-current configuration.

[0074] In accordance with at least one implementation, media, including cells (from a source such as a cell container, e.g. a bag) may be attached at attachment point 662, and fluid media from a media source may be attached at attachment point 646. The cells and media may be introduced into first fluid circulation path 602 via first fluid flow path 606. Attachment point 662 may be fluidly associated with the first fluid flow path 606 via valve 664, and attachment point 646 may be fluidly associated with the first fluid flow path 606 via valve 650. A reagent source may be fluidly connected to point 644 and be associated with fluid inlet path 642 via valve 648, or second fluid inlet path 674 via valves 648 and 672.

[0075] Air removal chamber (ARC) 656 may be fluidly associated with first circulation path 602. The air removal chamber 656 may include one or more sensors including an upper sensor and lower sensor to detect air, a lack of fluid, and/or a gas/fluid interface, e.g., an air/fluid interface, at certain measuring positions within the air removal chamber 656. For example, ultrasonic sensors may be used near the bottom and/or near the top of the air removal chamber 656 to detect air, fluid, and/or an air/fluid interface at these locations. Implementations provide for the use of numerous other types of sensors without departing from the spirit and scope of the present disclosure. For example, optical sensors may be used in accordance with implementations of the present disclosure. Air or gas purged from the CES 600 during portions of a priming sequence or other protocol(s) may vent to the atmosphere out air valve 660 via line 658 that may be fluidly associated with air removal chamber 656.

[0076] An EC media source may be attached to EC media attachment point 668 and a wash solution source may be attached to wash solution attachment point 666, to add EC media and/or wash solution to either the first or second fluid flow path. Attachment point 666 may be fluidly associated with valve 670 that may be fluidly associated with first fluid circulation path 602 via valve 672 and first fluid inlet path 642. Alternatively, attachment point 666 may be fluidly associated with second fluid circulation path 604 via second fluid inlet path 674 and second fluid flow path 684 by opening valve 670 and closing valve 672. Likewise, attachment point 668 may be fluidly associated with valve 676 that may be fluidly associated with first fluid circulation path 602 via first fluid inlet path 642 and valve 672. Alternatively, fluid container 668 may be fluidly associated with second fluid inlet path 674 by opening valve 676 and closing valve distribution 672.

[0077] In the IC loop, fluid may be initially advanced by the IC inlet pump 654. In the EC loop, fluid may be initially advanced by the EC inlet pump 678. An air detector 680, such as an ultrasonic sensor, may also be associated with the EC inlet path 684.

[0078] In at least one implementation, first and second fluid circulation paths 602 and 604 are connected to waste line 688. When valve 690 is opened, IC media may flow through waste line 688 and to waste or outlet bag 686. Likewise, when valve 692 is opened, EC media may flow to waste or outlet bag 686.

[0079] After cells have been grown in hollow fiber bioreactor 601, they may be harvested via cell harvest path 697. Here, cells from hollow fiber bioreactor 601 may be harvested by pumping the IC media containing the cells through cell harvest path 697, with valve 698 open, into cell harvest bag 699.

[0080] Various components of the CES 600 may be contained or housed within a machine or housing, such as cell expansion machine 202 (FIGS. 2 and 3), wherein the machine maintains cells and media at a predetermined temperature. It is further noted that, in implementations, components of CES 600 and CES 500 (FIG. 5) may be combined. In other implementations, a CES may include fewer or additional components than those shown in FIGS. 5 and 6 and still be within the scope of the present disclosure. In implementations, portions of CES 500 and 600 may be implemented by one or more features of the QUANTUM® Cell Expansion System (CES), manufactured by Terumo BCT, Inc. of Lakewood, CO.

[0081] In one specific implementation of using CES 600, hematopoietic stem cells (HSC's), e.g., CD34+ HSCs, may be expanded in an implementation of CES 600. In this implementation, HSC's (including CD34+ HSCs), which may be collected using a leukapheresis process or a manual process (e.g., umbilical cords), may be introduced into the bioreactor 601. The HSC's (including CD34+ HSCs) may be introduced into the bioreactor 601 through path 602.

[0082] In some implementations, the HSC's (including CD34+ HSCs) may be subjected to a selection process (e.g., a purification process) before introduction into bioreactor 601.

The process may involve the use of a centrifuge, purification column, magnetic selection, or chemical selection. Some examples of cell selection/ purification procedures include use of isolation columns from, for example, Miltenyi Biotec of Bergisch Gladbach, Germany. In one example, cord blood is first subjected to a cell selection process that selects for HSC's (including CD34+ HSCs) before the cells are introduced into the bioreactor 601. Other examples may utilize apheresis machines to deplete other cells that may be included with the HSC's (including CD34+ HSCs) when originally collected. For example, the HSC's may be sourced from cord blood, bone marrow, or peripheral blood. After initial collection, but before being introduced into the bioreactor 601, a volume of HSC's including CD34+ HSCs may be processed to deplete red blood cells, specific leukocytes, granulocytes, and/or other cells from the volume. These are merely some examples, and implementations of the present invention are not limited thereto.

[0083] In other implementations, the HSC's (including CD34+ HSCs) may be added directly to the bioreactor 601 after collection without any additional purification. For example, cord blood (with HSC's) may be added to the bioreactor. In addition to a number of proteins and other bioactive molecules, the cord blood may include HSC's (including CD34+ HSCs), red blood cells, platelets, granulocytes, and/or leukocytes.

[0084] It is noted that in some implementations, the HSC's may be added to bioreactor 601, after a priming step. As may be appreciated, the cells being expanded may not be adherent and therefore it may not be required that they adhere to the hollow fiber walls of bioreactor 601 for expansion/proliferation. In these implementations, it may be unnecessary to coat the inside of the hollow fibers with a coating to promote adhesion, e.g., fibronectin. In these implementations, the HSC's (including CD34+ HSCs) (purified or unpurified) may be introduced into the bioreactor 601 after a priming step and without a bioreactor coating step. If the cells were adherent cells, a coating step may be performed after the priming step and before introduction of the HSC's.

[0085] Once in the bioreactor 601, the cells may be exposed to growth factors, activators, hormones, reagents, proteins, and/or other bioactive molecules that may aid in the expansion of the cells. In one example, a co-culture cell line may have been previously grown/introduced, in the bioreactor 601, to optimize the conditions for growing the HSC's (including CD34+ HSCs). In one specific implementation, human mesenchymal stem cells (hMSC's) may be co-cultured with the HSC's (including CD34+ HSCs) to promote growth of CD34+ HSCs. Without being bound by theory, it is believed that MSC's may emit factors (e.g., SDF-1 factors) that interact with HSC's (e.g., CD34+ HSCs) and promote proliferation of these cells. In some implementations, use of the co-cultured hMSC's may involve a growing process that is performed initially, under conditions optimized for proliferating the hMSC's, before the HSC's (including CD34+ HSCs) are introduced into the bioreactor 601. The hMSC's may be derived in implementations from bone marrow, peripheral blood, cord cells, adipose tissue, and/or molar tissue.

[0086] In addition to co-culture cells, a supplement including one or more growth factors, activators, hormones, reagents, proteins, and/or other bioactive molecules may be added to bioreactor 601 to grow and expand the HSC's. The supplement may be added as a single volume addition or over a period of time (e.g., continuously, intermittently, or on a regular schedule). In one implementation, a combination of cytokines and/or other proteins, e.g., recombinant cytokines, hormones, may be included as part of the supplement. As one example, a supplement may include one or more of: recombinant human Flt3 ligand (rhFlt-3L), recombinant human stem cell factor (rhSCF), recombinant human thrombopoietin (rhTPO), recombinant human (rh) Glial-derived neurotrophic factors and/or combinations thereof. One example of a supplement that may be used with implementations is STEMCELL2MAXTM supplement (stemcell2MAX, Cantanhede, Portugal).

[0087] It is noted that in some implementations, the combination of factors may be included in the media in which the cells are suspended. For example, the HSC's may be suspended in media and introduced 1406 into the bioreactor in the media. In implementations, the media may include a combination of growth factors that aid in proliferation of the HSC's.

[0088] After the cells have been introduced into the bioreactor with the supplement, co-culture cells, and/or other material for expanding the cells, the cells are allowed to expand in bioreactor 1410. During the expansion, there may be materials that may be added or removed from bioreactor. As one example, additional proteins (e.g., cytokines) may be added to bioreactor 601. In some implementations, more than one protein or other bioactive agent may be used. The additional material may be added individually, at the same time, at different times, or may be combined and added in combination.

[0089] It is noted that some implementations may provide for adding material more directly into the bioreactor 501, such as through port 516 (FIG. 5). In other implementations, however, the materials may be added in a location, e.g., through path 606, so that the materials may be perfused more slowly into bioreactor 601.

[0090] In addition to materials for aiding in growing the HSC's (including CD34+ HSCs), the HSC's may also be fed, such as by addition of a media that may include a number of nutrients. In some implementations, the media may be commercially available media that may include serum. In other implementations, the media may be serum free and include other additives. The media may be modified by the addition of other materials, some non-limiting examples including salts, serum, proteins, reagents, bioactive molecules, nutrients. One example of media that may be used to feed the HSC's (including CD34+ HSCs) includes CELLGRO® serum free media (CellGenix, Freiburg, Germany).

[0091] In some implementations, while the co-culture cells are located in the IC space, feeding may occur in the EC space. Feeding through the EC space may, in implementations, reduce the amount of force that may be felt by the cells from circulating fluid in the IC space. Circulation of media in the EC space may, in implementations, provide sufficient nutrients for the expansion of the HSC's (including CD34+ HSCs).

[0092] As part of the expansion of the HSC's (including CD34+ HSCs), other conditions such as temperature, pH, oxygen concentration, carbon dioxide concentration, waste concentration, metabolite concentration may also be controlled in bioreactor 601. In some implementations, the flow rates of the EC side, e.g., path 604 may be used to control various parameters. For example, if it is desired to reduce waste or metabolite concentrations on the IC side, where the cells are growing, flow rate on the EC side may be increased to ensure that the waste and/or metabolites are removed from the IC side by migration through the hollow fibers from the IC side to the EC side.

[0093] After the CD34+ HSCs have been expanded, the cells may be removed from the bioreactor 601. The CD34+ HSCs may be collected in container 699. In implementations, the collected CD34+ HSCs may be administered to a patient to reestablish hematopoiesis. Some non-limiting examples including patients undergoing treatment for various cancers, e.g., leukemia, myelodysplasia, non-Hodgkin lymphoma, which may effect hematopoiesis. The cells may be administered with other compounds or molecules.

[0094] In some implementations, use of CES 600 may provide advantages in growing HSC's (including CD34+ HSCs) over conventional processes. For example, the use of hollow fibers allows close cell to cell communication, which may enhance the growth of the CD34+ HSCs to start and continue to proliferate. Also, the use of a hollow fiber bioreactor, such as bioreactor 601, may provide a large surface area for cell growth, which may yield a higher concentration or higher volume of CD34+ HSCs.

[0095] Further, the conditions in bioreactor 601 may be controlled using a number of different components of the CES 600, including IC flow rates and EC flow rates. Also, CES 600 provides various locations for the addition of materials, which allows more direct, or indirect, e.g., perfusion, of cytokines into bioreactor 601.

[0096] Additionally, CES 600 provides a closed system. That is, the steps for growing the CD34+ HSCs may be performed without direct exposure to the ambient environment, which may contaminate the cells, or be contaminated by the cells or materials used in growing the cells. It is also believed that some implementations may provide for using a smaller starting concentration of CD34+ HSCs for expansion, compared to other methods/systems. In these implementations, CD34+ HSCs may also be expanded to yield larger amounts than from other methods/systems. It is also believed that some implementations may provide for shortening the time for growing an effective dose of CD34+ HSCs.

[0097] FIG. 7 illustrates example components of a computing system 1100 upon which implementations of the present disclosure may be implemented. Computing system 1100 may be used in implementations, for example, where a cell expansion system uses a processor to execute tasks, such as custom tasks or pre-programmed tasks performed as part of processes, such as the process described above.

[0098] The computing system 1100 may include a user interface 1102, a processing system 1104, and/or storage 1106. The user interface 1102 may include output device(s) 1108, and/or input device(s) 1110 as understood by a person of skill in the art. Output device(s) 1108 may include one or more touch screens, in which the touch screen may comprise a display area for providing one or more application windows. The touch screen may also be an input device 1110 that may receive and/or capture physical touch events from a user or operator, for example. The touch screen may comprise a liquid crystal display (LCD) having a capacitance structure that allows the processing system 1104 to deduce the location(s) of touch event(s), as understood by those of skill in the art. The processing system 1104 may then map the location of touch events to user interface (UI) elements rendered in predetermined locations of an application window. The touch screen may also receive touch events through one or more other electronic structures, according to implementations. Other output devices 1108 may include a printer, speaker. Other input devices 1110 may include a keyboard, other touch input devices, mouse, voice input device, as understood by a person of skill in the art.

[0099] Processing system 1104 may include a processing unit 1112 and/or a memory 1114, according to implementations of the present disclosure. The processing unit 1112 may be a general purpose processor operable to execute instructions stored in memory 1114. Processing unit 1112 may include a single processor or multiple processors, according to implementations. Further, in implementations, each processor may be a multi-core processor having one or more cores to read and execute separate instructions. The processors may include general purpose processors, application specific integrated circuits (ASICs), field programmable gate arrays (FPGAs), other integrated circuits, as understood by a person of skill in the art.

[0100] The memory 1114 may include any short-term or long-term storage for data and/or processor executable instructions, according to implementations. The memory 1114 may include, for example, Random Access Memory (RAM), Read-Only Memory (ROM), or Electrically Erasable Programmable Read-Only Memory (EEPROM), as understood by a person of skill in the art. Other storage media may include, for example, CD-ROM, tape, digital versatile disks (DVD) or other optical storage, tape, magnetic disk storage, magnetic tape, other magnetic storage devices, as understood by a person of skill in the art.

[0101] Storage 1106 may be any long-term data storage device or component. Storage 1106 may include one or more of the systems described in conjunction with the memory 1114, according to implementations. The storage 1106 may be permanent or removable. In implementations, storage 806 stores data generated or provided by the processing system 104.

[0102] This disclosure provides methods of expanding cells (i.e., increasing the number of cells grown in culture). In particular, these methods are useful in the expansion of human hematopoietic stem cells (HSCs), including HSCs that express CD34 protein (CD34-positive HSCs, or CD34+ HSCs). In these methods, the CD34+ HSCs may be CD45+/CD34+ HSCs and/or CD133+CD38- progenitor cells. These methods advantageously expand HSCs many fold (for example, at least 50-fold) quickly and efficiently, while minimizing the differentiation of these HSCs.

[0103] Flow 1400 may be performed in embodiments to expand target cells, such as CD34+ HSCs in monoculture or co-culture. Flow 1400 starts at step 1404 and proceeds to step 1412 where expanded cells (e.g., CD34+ HSC's)) may be removed from a bioreactor.

[0104] Similarly, the cells to be expanded in these methods may be cells collected from a donor fluid (e.g., one or more blood components) that may include stem cells, CD34+

HSCs, T-cells, monocytes, and/or natural killer (NK) cells. In these methods, specific "target" cells within the donor fluids (e.g. CD34+ HSCs) may be expanded while other cells present in the donor fluid are removed or reduced in number.

[0105] These methods may include expanding the cells in culture on a membrane. Within these methods of cell expansion, the membrane may be useful in trapping, collecting, and/or otherwise holding cells. The membrane may be arranged in any form, such as a flat sheet, a filter matrix, a hollow fiber, any combination thereof, and/or any plurality thereof. In these methods, the membranes may comprise a coating on at least one surface of the membrane, wherein the coating comprises stromal cell-derived factor-1 (SDF-1), and fibronectin or isoforms, or functional equivalents thereof. A particularly useful membrane within the methods of this disclosure is a hollow fiber or a plurality of hollow fibers, as they appear within a hollow fiber bioreactor. Such hollow fibers contain an interior portion or surface within the lumen of the hollow fiber, and an exterior surface ("extracapillary" side or surface). The hollow fiber membrane may comprise a plurality of hollow fibers. An example of a hollow fiber may be as shown in the schematic representation of FIG. 8, depicting a length of the hollow fiber 800 and an end of the hollow fiber 802, with a lumen 804 and an extracapillary side 808 of the hollow fiber 800. As depicted in FIG. 8, the lumen surface 806 may have a coating 810.

[0106] In some examples of these methods, the membrane may be used in conjunction with a cell processing device. In one example, the cell processing device may be the SPECTRA OPTIA® apheresis system, COBE® spectra apheresis system, and the TRIMA ACCEL® automated blood collection system, all manufactured by Terumo BCT, of Lakewood, Colorado. After the cells are collected from a donor, the cells may be passed through the membrane to isolate target cells therefrom.

[0107] In some examples of these methods, the membrane may be used in conjunction with a cell expansion device. In one example, the cell expansion device may correspond to the Quantum® Cell Expansion System manufactured by Terumo BCT, of Lakewood, Colorado. After the target cells are isolated (e.g., as described above), the target cells may be expanded in the membrane to, for example, increase a number of the target cells contained therein.

[0108] Capture of small sized species, such as proteins or exosomes may be conducted on a continuous flow basis over the membrane. Diffusion dynamics may be effective in helping to transport these species to the membrane where they can be captured by their conjugate chemistry which has been deposited on the membrane. Transport to fiber walls may be further assisted by moderate ultrafiltration from the lumen side 906 to the extracapillary side of the hollow fiber 900 as illustrated in the schematic representation of FIG. 9 (arrows indicate direction of ultrafiltration flow). Ultrafiltration flow may occur through a coating 910 present on the lumen surface 906 of the hollow fiber 900. Flow may be stopped for segregating mixed cell populations as illustrated in the schematic representation of FIG. 10, wherein particles 1002 in the lumen of the hollow fiber 1000 are suspended in the lumen 1004 while flow through the lumen 1004 has stopped. When there is no flow, the particles 1002 (e.g., HSCs) may fall to the bottom 1006 of the hollow fiber 1000 as illustrated in the schematic representation of FIG. 11. At this point, target species (such as cells) may adhere to the surface(s) of the membrane and non-target species (such as cells or cellular debrix) may be washed from the membrane leaving target species contained on the membrane. In one example, when non-target species have been removed, a release mechanism (including but not limited to changing pH, changing temperature, displacing binder chemistry) may be used to release the association (such as a bond) between the membrane components and the target species (for example between an aptamer and a cell membrane antigen) leaving the target species in their native, unmodified state. For example, an aptamer may be cleaved by an appropriate nuclease to break the bond between an aptamer and a cell, to release the cell.

[0109] The membrane may comprise one or more coatings that are configured to attract, collect, and/or hold target cells, which may then be expanded. In the instance when the membrane is the hollow fibers of a hollow fiber bioreactor, the hollow fibers may comprise a coating on one or both of the lumen surface and the extracapillary surface of the hollow fibers. The coating provided in this disclosure may be a coating that is chemically linked to the membrane (e.g., through hydrophobic and hydrophilic interaction). In some examples, a base coating material may serve as a first coating layer, and a secondary coating material may serve as a secondary coating layer. These coating materials may be applied to a membrane sequentially or together. Examples of a first coating material may include fibronectin, vitronectin, any extracellular matrix (ECM) glycoprotein, collagen, enzyme, equivalents and/or combinations thereof, and/or any molecule or material that is capable of providing cellular adhesion to a membrane or other surface. Examples of a secondary coating material may include a soluble protein moiety, biotinylated molecules, an anti-biotin antibody, a biotin-binding and/or streptavidin-binding peptide, a streptavidin, an avidin, monoclonal antibodies, aptamers (e.g., aptamers targeted toward specific cell surface markers), cytokines (e.g., Interleukin (IL)-6 , IL-21), chemokines (e.g., stromal cell-derived factor (SDF)-1), equivalents and/or combinations thereof.

[0110] The coating may be applied in a single chemical operation. For instance, a first molecule (e.g., the first-part coating material) and a second molecule (e.g., the second-part coating material) may be conjugated outside of the membrane and then coated onto the membrane at the same time. When formed by coating membranes of this disclosure may be used to (1) make a selective bioreactor to expand cells; and/or (2) create a filter that can capture a specific target cell or molecule (such as any biotinylated molecule or cell).

[0111] In one example, the membrane may comprise one or more materials that promote cellular adhesion to at least one surface of the substrate. For example, the coating may comprise the dimeric glycoprotein fibronectin, or a functional equivalent of fibronectin, such as the many known isoforms of fibronectin created through alternative splicing of its pre-mRNA, or other proteins that contain the integrin-binding sequence, Arg-Gly-Asp (RGD) of fibronectin proteins that provides the primary cell adhesive activity of fibronectin.

[0112] Additional useful coatings may include one or more protein moieties. Such protein moieties may be selected to target specific target cells present within a donor fluid. For example, the protein moiety may be a chemokine, such as stromal cell-derived factor-1 (SDF-1), which may be used to enhance collection of CD34+ HSCs from a donor fluid (e.g., when compared to an uncoated membrane or a membrane coated only with fibronectin). Another useful protein moiety in these coatings may be interleukin-21 (IL-21). Another useful protein moiety in these coatings may be the combination of SDF-1 and IL-21. Another useful protein moiety in these coatings may be the combination of fibronectin and SDF-1, as depicted in FIG. 12, wherein a coating 1210 comprising the combination of combination of fibronectin and SDF-1 is associated with the lumen surface of the hollow fiber 1200. Additional coatings of the membrane may be selected to target, collect, and/or hold the same or different cells from within a donor fluid.

[0113] In these methods, the membrane may be coated with a mixture of fibronectin and a soluble protein moiety as illustrated in the schematic representation of FIG. 5.

[0114] As depicted in FIG. 13, in these methods, a plurality of cells 1302 (such as a suspension of HSCs) may be introduced into the hollow fiber membrane 1300 having a coating 1310 on the lumen surface 1306.

[0115] In some implementations, the coated membrane may be coated with a mixture of fibronectin and a soluble protein moiety to capture biotinylated molecules, such as streptavidin, avidin, and/or anti-biotin antibodies and/or functional equivalents thereof.

[0116] As illustrated in the schematic representation of FIG. 15, in some examples, the lumen surface 1506 of the hollow fiber 1500 may have a coating 1510, which may be, for example, a coating comprising biotinylated molecules. In one example, this type of membrane coating may allow for target species 1502 (such as HSCs) within a suspension of non-target species 1503 (such as red blood cells) to be captured. The coated membrane may be used to isolate or captures target cells from a mixed population of cells. As depicted in FIG. 16, target cells 1502 may be captured by, for example, a protein moiety present in the coating 1510 on the lumen surface 1506 of the hollow fiber 1500. Following flushing 1550 of the hollow fiber 1500, non-target species 1503 are removed from the lumen 1504 of the hollow fiber 1500, while target species 1502 remain bound to the lumen surface 1506 of the hollow fiber 1500.

[0117] In these methods, a plurality of cells are directed into contact with a membrane, which may be a coated membrane of this disclosure, and expanded while in contact with the membrane. In the instance which a hollow fiber membrane is used in these methods, the plurality of cells may be introduced 1406 into hollow fibers of a hollow fiber bioreactor, wherein the hollow fibers each comprise an interior lumen and an extracapillary side, as described above. The plurality of cells may be first purified by various means prior to being directed into contact with the membrane. Alternatively, the plurality of cells may be directed into contact with the membrane without any initial purification, such as direct from collection from a donor source of cells (e.g. a collection of peripheral blood, or bone marrow, or cord blood (CB)), which may include introducing the plurality of cells into a plurality of hollow fibers without any prior purification. The cells may be directed into contact with the membrane and then left in that position to associate with the membrane, before additional circulation or movement against the membrane to “seed” additional cells on the membrane or remove residual cells or cellular debris from the membrane. When the membrane includes the hollow fibers of a hollow fiber bioreactor, this procedure may advantageously include circulating, with a pump, the plurality of cells within the lumen of the hollow fibers, and then stopping the pump to allow a portion of the plurality of cells to attach to a first portion of the lumen of the hollow fibers, and then rotating the hollow fiber bioreactor 180 degrees from an initial position before again circulating, with the pump, the plurality of cells within the lumen of the hollow fibers, and then stopping the pump to allow a portion of the plurality of cells to attach to a second portion of the lumen of the hollow fibers.

[0118] The cells may be expanded 1410 by exposing 1408 the cells in the hollow fibers to growth conditions. The growth conditions may include exposing the cells to one or more of a cell growth media, for example, by circulating a cell growth media through the lumen of hollow fibers of a hollow fiber bioreactor and/or through the extracapillary side of the hollow fibers. Alternatively or additionally, the growth conditions may comprise exposing the cells to one or more growth factors. Useful growth factors may include FMS-like Tyrosine Kinase 3 Ligand (Flt-3L), Stem Cell Factor (SCF), thrombopoietin (TPO), glial cell-derived neurotrophic factor (GDNF), interleukin-3 (IL-3), interleukin-6 (IL-6), IL-21, SDF-1, or combinations thereof. In the instance GDNF is present in the growth media, it may be particularly useful at a concentration of 0.5% to 2% weight per volume, such as at a concentration of about 10 ng/mL in the growth media.

[0119] Within these methods that use the membrane of hollow fibers of a hollow fiber bioreactor, a first media may be used in the lumen of the hollow fibers and a second media maybe used in contact with the extracapillary side of the hollow fibers. In these methods, the media in the lumen may be concentrated in at least one component relative to the concentration of the same component on the extracapillary side of the hollow fibers. In these methods, the concentrated component may be GDNF, SR-1, SCF, TPO, Flt-3L, IL-3, IL-6, SDF-1, fibronectin, or combinations thereof. In these methods, the concentrated component may be concentrated at least five-fold, or at least ten-fold.

[0120] Another useful factor for expanding the cells may include an aryl hydrocarbon receptor antagonist, such as StemRegenin 1 (SR1) or UM171, which was developed at the University of Montreal and which is in clinical development for cell therapy by ExcellThera, Inc.

[0121] The coating may be used to provide a specialized environment for cell culture (e.g., when the coating comprises a base coating material, such as fibronectin, and a secondary coating material comprising a soluble protein moiety, such as SDF-1, IL-21). Accordingly, this disclosure provides compositions useful for expanding CD34+ HSCs. These compositions may comprise at least one of glial cell line-derived neurotrophic factor (GDNF), and an aryl hydrocarbon receptor (AHR) antagonist (such as SR-1). These compositions may also include at least one of SCF, TPO, Flt-3L, IL-3, IL-6, SDF-1, and fibronectin. In these compositions, GDNF may be is present at a concentration of 0.5% to 2% weight per volume, or at a concentration of at least 10 ng/mL. In these compositions, fibronectin and SDF-1 may be immobilized on a cell culture surface, such as a semi-permeable membrane. These compositions may increase levels of BCL2 and inhibit HSC differentiation.

[0122] The coated membranes of this disclosure may be used to provide a specialized environment to capture biotinylated molecules, such as streptavidin, avidin, anti-biotin, (e.g., when the coating comprises a first coating material, such as fibronectin, and a secondary coating material comprising a biotin capture moiety, such as biotinylated molecules, aptamers targeted toward specific cell surface markers, or soluble moieties such as a cytokine (e.g., IL-6)).

[0123] At least one benefit to the chemical coating described herein is the ability to manufacture membranes (e.g., hollow fiber membranes) having the coating in a sterile environment. A sterile package including the chemically coated, and sterilized, coated membrane may be opened and ready to use after removing the membrane from the package (e.g., without requiring further processing).

[0124] In one example, the Quantum® Cell Expansion System bioreactor hollow fiber membrane (HFM) may be coated with a coating material comprising streptavidin-fibronectin. This coating material may be used, for example, to select or isolate specific cell types when subsequently coupled with biotinylated cell-specific monoclonal antibodies (mAbs).

[0125] In some examples, a fibronectin-streptavidin foundation may be used as the coating material for the attachment of biotinylated molecules to functionalize the surface of a polyethersulfone HFM bioreactor, or preparatory columns, for cell selection. Fibronectin may bind to the polyethersulfone HFM in the Quantum® Cell Expansion System bioreactor through the adherence and expansion of adherent cells such as mesenchymal stromal/stem cells (MSCs), fibroblasts and aortic endothelial cells. This fibronectin-streptavidin conjugation may take advantage of a high affinity of streptavidin binding for biotin. While considering available protein coupling biochemistries, it may be important to keep the protocols direct and efficient with minimal residue or reactants to accommodate their adaption in the manufacturing of cell therapy products. In one example, fibronectin-streptavidin mixture or conjugate may be mixed and/or linked, which will allow the HFM bioreactor or column with biotinylated cytokines, chemokines, and/or other ligands to facilitate cell selection and/or expansion. Other affinity separations of biomolecules may also be used. In any case, this protein-protein conjugation can be viewed as a platform for affinity processes associated with cell therapy which uses available technology.

[0126] In these methods, a mixture of fibronectin and streptavidin may be used as the coating material for the coated membrane. This process may include reconstitution of lyophilized fibronectin and streptavidin (e.g., in a ratio of 1:3.3 by mass) in water at ambient temperature for approximately 30 minutes. After the conjugation of fibronectin-streptavidin, the mixture volume may be brought up to 100 mL with phosphate buffered saline w/o Ca2+-Mg2+ and introduced into the Quantum® Cell Expansion System using the “Coat Bioreactor” task for a sufficient period of time (e.g., 8 hours). After the bioreactor coating, excess unbound conjugated protein may be washed out and a selected biotinylated molecule, for example, cytokine (interleukin or growth factor), epitope, ligand, monoclonal antibody, stains, or aptamer, may be introduced into the Quantum® Cell Expansion System bioreactor using the “Coat Bioreactor” task for coupling to the fibronectin-streptavidin coating. Once complete, the resulting fibronectin-streptavidin-bioconjugate protein may be ready for use in cell selection or cell signaling (including differentiation) applications. Other applications may include the coating of preparatory HFM columns or matrixes which could be used for cell selection or differentiation prior to the introduction of cells into the Quantum® Cell Expansion System.

[0127] In these methods, recombinant or semi-synthetic fibronectin or fibrinogen may be substituted for plasma-derived fibronectin. Extracellular matrix proteins such as fibronectin may bind to the polyethersulfone hollow fiber membrane by virtue of polarity and hydrogen bonding. Fibronectin has a naturally adhesive nature due to its glycoprotein structure and specific domains which may allow fibronectin to bind to both polyethersulfone and cell membrane integrins.

[0128] In one example, the covalent coupling of fibronectin to streptavidin, using a similar mass ratio as outlined above, may be achieved using a streptavidin conjugation kit. This kit may make use of a specific linkage modifier and quencher chemistry to generate a covalent linkage between fibronectin and streptavidin in a time period of 30 minutes to 24 hours, and in some implementations in a time period of 3 hours to 15 hours. In some examples, the time to generate a covalent linkage between fibronectin and streptavidin may be approximately 4 hours, plus or minus 30 minutes. The affinity of the chosen biotinylated molecule to streptavidin, in the covalent coating method, may be similar to the affinity of the biotinylated molecule in the fibronectin-streptavidin mixture coating method. One advantage of the covalent approach may include an improved stability of the fibronectin-streptavidin coupling.

[0129] The coupling of streptavidin-biotinylated molecules to fibronectin using a molar ratio, for example, of 1:3 (fibronectin:streptavidin) may be useful. In some examples, the coupling of the fibronectin-streptavidin biotinylated molecules to the HFM bioreactor may be a two-step process. This conjugation coating chemistry may be a platform for binding an array of biotinylated molecules for cell selection, stimulation, expansion, or differentiation.

[0130] Fibronectin-streptavidin protein conjugate may be selected as an adhesion molecule for the Quantum® Cell Expansion System bioreactor. Coupling biotinylated cell-specific mAbs or protein epitopes to the fibronectin-streptavidin conjugate may exploit the high affinity of streptavidin for biotin at a specific ratio of up to and including 1:4 with an approximate disassociation constant of $Kd = 10^{-14}$ to 10^{-15} M. Examples of biotinylated antibodies or epitopes which are cell-specific may include anti-CD3 mAb for parent T cells, anti-CD4/CD25 mAb for human T-reg cells, anti-CD8 mAb for human T-effector cells, anti-CD34 mAb for hematopoietic stem cells, or anti-CD56 mAb for NK cells. The streptavidin-biotin linkage may comprise a strong non-covalent linkage and, as such, this functional specificity can be used to select for virtually any cell type by simply changing the specificity of the biotinylated mAb conjugate. In addition, it is also possible that the reverse approach could be utilized where biotinylated-fibronectin would couple with streptavidin-cell specific mAb, which could be used to select cells of interest. If the first approach were used, then the biotinylation of mAbs, with the small biotin molecule (m.w. 244.3 Daltons), is less likely to affect mAb binding or cell antigen recognition. Secondly, the net negative charge and lack of glycosylation streptavidin may serve to minimize the non-specific binding of cells. This concept can leverage the highly specific interaction and versatility of the streptavidin-biotin interaction to provide a better adhesion system. In some examples, cells may be enzymatically separated from the streptavidin-fibronectin-biotin-mAb-cell complex by enzymatically cleaving the DNase-sensitive linker.

[0131] Accordingly, this disclosure also provides a coated membrane, and methods of making and using the same. These coated membranes may be hollow fibers, including those hollow fibers used in hollow fiber bioreactors. These coated hollow fiber membranes may include lumen surface and an extracapillary surface and have a first coating on at least one of the lumen surface and an extracapillary surface. The first coating may comprise a material that promotes cellular adhesion to at least one of the lumen surface and an extracapillary surface. The second coating on at least one of the lumen surface and an extracapillary surface, may comprise a soluble protein moiety. In these coated hollow fiber membranes, the first coating may comprise fibronectin. In these coatings, the second coating may comprise at least one of a cytokine, an aptamer, a chemokine (for example, SDF-1 or IL-21), a monoclonal antibody, streptavidin, avidin, a biotinylated molecule, and an anti-biotin antibody or a functional fragment thereof. These membranes may be composed of a material comprising polysulfone or polyethersulfone.

[0132] In these coated hollow fiber membranes, the amount of the fibronectin coating the hollow fiber may be 0.001 $\mu g/cm^2$ to 2 $\mu g/cm^2$, or may be 0.01 $\mu g/cm^2$ to 1.0 $\mu g/cm^2$, or may be 0.10 $\mu g/cm^2$ to 0.50 $\mu g/cm^2$, or may be 0.20 $\mu g/cm^2$ to 0.40 $\mu g/cm^2$, or may be 0.23 $\mu g/cm^2$ to 0.24 $\mu g/cm^2$. In these coated hollow fiber membranes, the amount of the SDF-1 coating the hollow fiber may be 0.001 ng/cm^2 to 0.30 ng/cm^2, or may be 0.01 ng/cm^2 to 0.10 ng/cm^2, or may be 0.05 ng/cm^2 to 0.09 ng/cm^2, or may be 0.075ng/cm^2.

[0133] This disclosure also provides methods of forming a coated hollow fiber membrane. These methods include providing a hollow fiber membrane having a lumen surface and an extracapillary surface, and applying a first coating onto the lumen surface of the hollow fiber membrane, and applying a second coating onto the lumen surface of the hollow fiber membrane. In these methods, the first coating may comprise a material that promotes cellular adhesion to at least one of the lumen of the hollow fiber membrane and the extracapillary surface of the hollow fiber membrane (such as fibronectin) and the second coating may comprise a soluble protein moiety, such as one or more of one of a cytokine, an aptamer, a chemokine, a monoclonal antibody, streptavidin, avidin, a biotinylated molecule, and an anti-biotin antibody or a functional fragment thereof. In these methods, applying the first coating and the second coating material may comprise conjugating a first coating material and a second coating material into a conjugate apart from the hollow fiber membrane and coating the conjugate onto the lumen surface of the hollow fiber membrane. These methods may include applying the first coating onto the extracapillary surface of the hollow fiber membrane and/or applying the second coating onto the extracapillary surface of the hollow fiber membrane. In these methods, the first coating may be fibronectin, and the second coating may be SDF-1 or interleukin-21 IL-21.

[0134] As described herein, the bioreactor (e.g., the HFM, the hollow fiber device, and/or the hollow fibers) may be coated sequentially. Sequentially coating the bioreactor may provide enhanced exposure to the SDF-1 moiety over time. For instance, in accordance with an example protocol, on Day -2 (e.g., two days before seeding): the bioreactor HFM may be coated with fibronectin (e.g., using the “Coat Bioreactor” task described above), on Day -1 (e.g., one day before seeding): the bioreactor HFM may be coated with SDF-1 (e.g., using the “Coat Bioreactor” task described above), and on Day 0 (e.g., the day of seeding): the bioreactor may be seeded with CB-derived CD34+ HSCs. In some examples, each coating may take between 8 hours and 24 hours to complete.

[0135] In these methods, the cells in contact with the membrane (such as cells within the lumen of a plurality of hollow fibers) may be expanded by growing in a monoculture (i.e., substantially in the absence of other cell types) or in a co-culture (i.e., in the presence of other cell types). For example, in these methods, CD34+ HSCs expanded in hollow fibers may be expanded in monoculture, wherein no additional cell type is co-cultured with the CD34+ HSCs in the hollow fibers. In these methods in which CD34+ HSCs are expanded in hollow fibers in monoculture, the hollow fibers may comprise a coating comprising SDF-1 and fibronectin on at least one of the lumen surface and the extracapillary surface of the hollow fibers. In these methods, CD34+ HSCs are advantageously expanded in the presence of SDF-1 and fibronectin without the need for other cells in co-culture.

[0136] In these methods, the plurality of cells (such as CD34+ HSCs) may be expanded by growing in co-culture. CD34+ HSCs may be expanded in co-culture with mesenchymal stem cells. In these methods, cells to be grown in co-culture (such as mesenchymal stem cells) may be introduced into the hollow fibers before introducing the plurality of cells for expansion into the hollow fibers. The cells to be grown in co-culture (such as mesenchymal stem cells) may be grown in monoculture in the hollow fibers (such as by exposing the mesenchymal stem cells in the hollow fibers to growth conditions) before introducing the plurality of cells (such as CD34+ HSCs) to be expanded. Alternatively or additionally, the plurality of cells (such as CD34+ HSCs) to be expanded may first be grown in co-culture (such as in co-culture with mesenchymal stem cells) in a static growth chamber (such as traditional cell culture wells or flasks) before removing all or a portion of the plurality of cells (such as CD34+ HSCs) to be expanded from the static growth chamber and introducing the plurality of cells from the static growth chamber into the hollow fibers.

[0137] In these methods, the expansion of the cells may be advantageously sufficient to expand a plurality of cells comprising CD34+ HSCs obtained from a single unit of blood or tissue to a plurality of expanded cells sufficient for at least one engraftment procedure for a human recipient. In these instances, the single unit of blood may be cord blood, or the single unit of tissue may be bone marrow.

[0138] In these methods, the expanded cells comprising CD34+ HSCs may have at least 90% viability after expansion. In these methods, the expanded cells comprising CD34+ HSCs may be expanded at least 50-fold.

[0139] In some examples, this disclosure provides a method and device for the isolation of a target species, for example a target cell or a target molecule, from a mixed population of non-target species. Isolation of a target cell from a mixed population of cells may be used to describe the method and device. A hollow fiber device similar to a hemodialyzer may be used in the cell isolation procedures. As described above, the intracapillary (lumen) walls of hollow fibers may be coated such that a specific binding reagent (e.g., coating materials) is uniformly attached to the intracapillary surface of the hollow fiber device. Additionally or alternatively, the extracapillary walls of the hollow fibers may be treated with a binding reagent, increasing a surface area (e.g., for molecular capture).

[0140] The binding reagent may, for example, correspond to a monoclonal antibody (mAb) or a sequenced aptamer. The binding reagent may be selected such that the binding reagent has a specificity for a receptor molecule on the surface of the target cell to be isolated. For instance, if T-cells are to be separated from a mononuclear cell (MNC) collection, a binding reagent with specificity for the CD3, CD4, CD8, and/or a combination of T-cell markers may be affixed (e.g., applied, coated, deposited) to the intracapillary surface and/or the extracapillary surface of a hollow fiber forming the membrane. One example of a method of isolating T-cells from the mixed cell population may include attaching antibodies or aptamers to a cell prior to introduction to the membrane and then passed over a streptavidin-coated membrane, such as a streptavidin-coated hollow fiber. In another example, a counter-flow confinement (CFC) approach is used, wherein a collection of cells may be flowed into the lumen side of the hollow fiber membrane. Once the cells are contained within the lumen side of the membrane, counterflow may be minimized to a level sufficient to retain the cells within the fibers. Once target cells are bound to the lumen surface, both longitudinal flow (lumen inlet header to lumen outlet header) and ultrafiltration flow may be used to remove unbound cells from the lumen of the hollow fiber.

[0141] In some examples, a release agent may be used to facilitate detachment of target cells from their binding sites (e.g., facilitating target cell harvest). The release agent may be flowed either longitudinally or with ultrafiltration or with both.

[0142] Although examples may be described herein in conjunction with a hollow fiber device (e.g., a bioreactor or other device comprising coated membranes arranged as hollow fibers), it should be appreciated that any membrane capable of receiving a coating can be used. For instance, any one or more of the following devices may be used to receive the various coatings and/or perform the methods described herein: large surface area hollow fiber device, a dialyzer (e.g., hemodialyzer), a cell-capture column (e.g., magnetic cell sorting, a magnetic column apparatus), a polysulfone membrane filter device, a cell processing system, and the like.

[0143] In these methods, at least a portion of the plurality of expanded cells may be removed 1412 from the membrane (such as the hollow fibers of a hollow fiber bioreactor). The expanded cells may then be stored, or used for transplantation or administration within other therapeutic procedures for a patient, such as a cancer treatment protocol. In these methods, administering the plurality of expanded cells to a patient may reconstitute hematopoiesis in the patient.

[0144] Human leukocyte antigen (HLA)-8-allele matched cord blood (CB) transplantation is an allogeneic procedure for the treatment of certain hematological malignancies, hemoglobinopathies, and autoimmune disorders. CB-derived $CD34^+$ stem cells and progenitor cells may be selected for hematopoietic reconstitution because of their increased capacity for self-renewal and proliferation, longer telomeres, and lower incidence of graft vs. host disease (GVHD) through a lower frequency of alloreactive T cells along with their ability to achieve rapid engraftment in hematological transplant recipients. However, one of the challenges in this setting, is to provide a sufficient number of T cell-depleted hematopoietic stem and progenitor cells which may be necessary to support mixed allogeneic hematopoietic stem cell transplantation (HSCT). Only about 4%-5% of the cord blood units stored in CB banks contain a sufficient number of CD34+ HSCs for single unit grafts ($\geq 1.05 \times 10^7$ CD34+ HSCs) or for double unit grafts ($\geq 1.40 \times 10^7$ CD34+ HSCs) for 70 kg patients.

[0145] Methods to expand cord blood-derived CD34+ HSCs, in either co-culture with mesenchymal stromal cells or with small molecules in combination with various cytokine supplements, frequently rely on inoculums of 4-6 x 10^6 or more CD34+ HSCs from cord blood units (CBUs). In some implementations (e.g., to extend the range of stored CBUs), a monoculture expansion protocol is provided for low initial seeding of 2 x 10^6 preselected cord blood-derived CD34+ HSCs in a cell processing system (e.g., the Quantum® cell expansion system's perfusion-based, 2-chambered, semi-permeable hollow fiber membrane (HFM) bioreactor) using a primary cytokine cocktail comprised of recombinant human-stem cell factor (SCF), -thrombopoietin (TPO), -fms-like tyrosine kinase 3 ligand (Flt3L), -interleukin 3 (IL-3), and -interleukin 6 (IL-6) at one-tenth of the manufacturer's recommended concentration. This cytokine cocktail may be further supplemented with recombinant human glial cell-derived neurotrophic factor (rhGDNF) to, for instance, maintain cell viability and combined with the aryl hydrocarbon receptor (AHR) antagonist SR-1. GDNF may upregulate the expression of the anti-apoptotic gene *BCL2* in human CB- CD34+ cell progenitors and SR-1 may limit HSC differentiation during CD34+ HSC expansion when implemented with other HSC cytokines. The proximity of mesenchymal stromal cells (MSCs) and hematopoietic stem and progenitor (HSPCs) in the bone marrow sinusoids, coupled with the perivascular support of HSPCs by SCF from CD146+ MSCs, may contribute to their inclusion in hematopoietic co-culture processes. However attractive, the co-culture of MSCs and HSPCs adds complexity, time, and potential variability to the stem cell and progenitor expansion process. Even so, MSC/HSPC co-culture may provide alternative production strategies in CB-derived CD34+ HSCs. Automating the hematopoietic cell and progenitor expansion process may provide a dependable quantity of selected cells for therapeutic indications.

[0146] Moreover, the Quantum® System may support the expansion of both adherent MSCs as well as suspension CD3+ T cells and Regulatory T cells with a perfusion-based HFM bioreactor. In the CB-derived CD34+ cell expansion method described herein, the intercapillary (IC) HFM lumen of the bioreactor may be coated with a mixture of human fibronectin (Fn) and the chemokine stromal derived factor 1 (SDF-1) prior to cell seeding in order to mimic the stimulatory and homing effects of bone marrow-derived or Wharton's Jelly-derived mesenchymal stromal cells. The preselected CB-derived CD34+ HSCs may be subsequently propagated under suspension culture conditions, and allowed to adhere to the coated-HFM IC-surface during this process, for example, to engage with the Fn-SDF-1 modified surface. In some cases, immobilized SDF-1 may be required to develop integrin-mediated cell adhesion of CD34+ HSCs by VLA-4 integrin to murine endothelial cells. In this context, hydrogel immobilization of SCF and SDF1α along with the incorporation of the PEG-RGD integrin recognition sequence onto the cell culture surface recapitulates certain aspects of the bone marrow microenvironment. The implementations and examples described herein provide expanding CB-derived CD34+ HSCs with a modified extracellular matrix protein.

[0147] In one example, a method and/or system for the automated monoculture expansion of CB-derived HSCs and progenitor cells beginning with mixed, positively selected CB-derived CD34+ HSCs is provided. These cells may be resuspended in serum-free medium and supplemented with a defined hematopoietic cytokine cocktail and expanded under a programmed, but modifiable, perfusion protocol for a period of 8 days, for example, to minimize T cell differentiation in the Fn-SDF-1 coated HFM bioreactor system. Quantum® System-expanded CB-derived CD34+ HSCs may generate a sufficient quantity of cells to support both single and double unit minimal CD34+ dose equivalency while conserving the CD34+ phenotype and with a minimal frequency of lymphocytes. Furthermore, these CB-derived expanded progenitor cells may demonstrate their ability to differentiate into mature hematopoietic colony forming units (CFUs) under methylcellulose assay conditions.

[0148] In an example implementation, three master lots of cord blood derived, preselected, mixed CD34+ HSCs may be expanded in an about 2.1m^2 HFM bioreactor with an about 124 mL perfusion-culture volume and harvested using an automated suspension cell protocol. Cells may be introduced into the intracapillary loop (e.g., the IC loop) of the HFM bioreactor through a defined perfusion protocol and maintained within the lumen of the bioreactor with a custom counter-flow fluidics program.

[0149] As noted above, the membranes of this disclosure may be used to effectively create a membrane that can capture a specific target cell or molecule. Thus, this disclosure also provides methods of capturing cells. Flow 2400 may be performed in embodiments to capture cells, such as CD34+ HSCs. Flow 2400 starts at step 2404 and proceeds to step 2414 where captured target cells (e.g., HSC's) may be removed from a bioreactor. These methods include introducing 2406 a mixture of target species, such as cells or molecules, and non-target species onto a membrane of this disclosure (such as into hollow fibers of a hollow fiber bioreactor wherein the hollow fibers each comprise an interior lumen and an extracapillary side). As described in detail above, these membranes comprise a coating on at least one surface of the membrane comprising at least one of a material that promotes cellular adhesion, and a protein moiety. In the instance of using a hollow fiber membrane, one or both of the lumen surface and the extracapillary surface of the hollow fibers may be coated with the material that promotes cellular adhesion and/or a protein moiety.

[0150] The mixture of species in contact with the membrane may be exposed 2408 to conditions that enhance the association of the target species with the membrane (i.e., “capture conditions”) 2410. Examples of capture conditions may include changes in pH, temperature, tonicity, and/or the addition or subtraction of compounds that enhance the association of the target species with the membrane. The implementation of the capture conditions may effectively capture at least a portion of the target cells on a surface of the membrane (such as at least one of the lumen and the extracapillary surface of hollow fibers). Thereafter, at least a portion of the non-target species may be flushed 2412 from the membrane (such as from the lumen of hollow fibers). In these capture methods, the target species may be, for example, CD34+ HSCs and the non-target species may be, for example, additional cell types or cellular debris or blood proteins.

[0151] In these capture methods, the coating material on the membrane that promotes cellular adhesion to a surface of the membrane may comprise fibronectin. In these capture methods, the protein moiety may be at least one of stromal cell-derived factor-1 (SDF-1), interleukin-21 (IL-21), streptavidin, avidin, and anti-biotin antibodies or functional fragments thereof. In these capture methods, the coating may comprise fibronectin and SDF-1. In these methods of capturing target cell species (such as CD34+ HSCs), after flushing at least a portion of the non-target cells from the membrane, the captured target cells may then be expanded, for example, by changing the media and/or other conditions at the membrane to enhance growth and expansion of the captured cells, such as CD34+ HSCs. These capture methods may include removing at least a portion of the captured target species (such as CD34+ HSCs) from the membranes. These captured species may be removed 2414 from the membrane after capture of the target species and flushing to remove non-target species, or after the target cell species have been expanded after capture, as described above.

[0152] This disclosure also provides methods of capturing cells, using the interaction of biotin and avidin. These methods include introducing a mixture of target species and non-target species into hollow fibers. In these methods, the hollow fibers each comprise an interior lumen and an extracapillary side, and the hollow fibers may comprise a coating on at least one of the lumen surface and the extracapillary surface of the hollow fibers. In these methods, the coating may comprise at least one of streptavidin, avidin, a biotinylated molecule, and an anti-biotin antibody or a functional fragment thereof. In these methods, the target and/or non-target species may be cells (e.g., HSCs) or molecules. In these methods, the mixture of target and non-target species may be exposed to capture conditions, to capture at least a portion of the target species on at least one of the lumen and the extracapillary surface of the hollow fibers. At least a portion of the non-target species may then be flushed from the hollow fibers. In these methods, the target cells introduced into the hollow fibers may comprise biotinylated aptamers or biotinylated antibodies that bind to the coating on the at least one of the lumen surface and the extracapillary surface of the hollow fibers.

[0153] These methods in which the target species are cells may include, after flushing at least a portion of the non-target cells from the hollow fibers, the portion of the target cells that are captured on a surface of the hollow fibers may be exposed to growth conditions to expand the portion of the target cells captured in the hollow fibers thereby generating a plurality of expanded target cells. In these methods, after capture of the target cells and flushing of the non-target cells, at least a portion of the captured target cells may be removed from the hollow fibers.

[0154] This disclosure also provides methods of expanding cells by perfusion in a cell expansion system. These methods may include coating a hollow fiber bioreactor with a first fluid, wherein the first fluid comprises a signaling factor and/or a coating factor. A plurality of cells may be introduced into the hollow fiber bioreactor, wherein the hollow fiber bioreactor comprises a hollow fiber membrane. The plurality of cells may be exposed to a second fluid, wherein the second fluid comprises a plurality of growth factors. The plurality of cells in the hollow fiber bioreactor may be grown in monoculture or in coculture. In these methods, the first fluid may comprise at least one of fibronectin and SDF-1. In these methods, the fibronectin and the SDF-1 may be mixed together prior to coating the hollow fiber bioreactor. In these methods, the hollow fiber bioreactor may be coated sequentially by coating the hollow fiber bioreactor with the fibronectin and then coating the hollow fiber bioreactor with the SDF-1. In these methods, the hollow fiber bioreactor may be coated sequentially by coating the hollow fiber bioreactor with the SDF-1 and then coating the hollow fiber bioreactor with the fibronectin. In these methods, an amount of the fibronectin used to coat the hollow fiber bioreactor may be 0.001 $\mu g/cm^2$ to 2 $\mu g/cm^2$, or 0.01 $\mu g/cm^2$ to 1.0 $\mu g/cm^2$, or 0.10 $\mu g/cm^2$ to 0.50 $\mu g/cm^2$, or 0.20 $\mu g/cm^2$ to 0.40 $\mu g/cm^2$, or 0.23 $\mu g/cm^2$ to 0.24 $\mu g/cm^2$. In these methods, an amount of the SDF-1 used to coat the hollow fiber bioreactor may be 0.001 ng/cm^2 to 0.30 ng/cm^2, 0.01 ng/cm^2 to 0.10 ng/cm^2, or 0.05 ng/cm^2 to 0.09 ng/cm^2, or 0.075ng/cm^2.

[0155] In these methods, the second fluid may comprise GDNF. In these methods, an amount of the GDNF in the second fluid may be 0.001 ng/mL to 40.0 ng/mL, or 0.01 ng/mL to 20 ng/mL, or 0.10 ng/mL to 15 ng/mL, or 1.0 ng/mL to 15 ng/mL, or 5.0 ng/mL to 15 ng/mL, or 10 ng/mL.

[0156] In these methods, the plurality of growth factors may comprise at least one of SCF, TPO, Flt-3L, IL-3, and IL-6. In these methods the second fluid may comprise StemRegenin (SR-1). In these methods an amount of the SR-1 in the second fluid may be 0.001 μM to 3.0 μM, or 0.01 μM to 2.0 μM, or 0.10 μM to 1.0 μM, or 0.75 μM.

[0157] In these methods, prior to introducing the plurality of cells into the hollow fiber bioreactor, hollow fiber bioreactor may be coated for a predetermined time period with a mixture of 5 mg of human plasma-derived fibronectin or 0.23-0.24 μg/cm^2 of fibronectin and recombinant human Stem Cell Derived Factor 1 (SDF-1) at 0.075 ng/cm^2. In these methods, the predetermined time period is 4.0 hours to 16.0 hours, or 8.0 hours to 12.0 hours.

EXAMPLES

[0158] **Example 1. Short Expansion Strategy for Cord Blood-Derived CD34+ HSCs in the Quantum® System**

[0159] Human cord blood-derived CD34+ hematopoietic stem cells (HSCs) expanded for 8 days or less engrafted more successfully in a humanized, immunodeficient murine model than cells expanded for greater than 8 days. Expanding cord blood-derived CD34+ HSCs for 8 days or less resulted in BALB/C-RAG2 null IL-2r-gamma null murine model humanized mice (Clinical Immunology, 140:102-116, 2011) displaying more consistent human hematopoietic and lymphoid engraftment.

[0160] Implementations provide for reducing, or shortening, the time period(s) for the expansion of cells, e.g., CD34+ HSCs and/or CB-CD34+ HSCs, while improving, for example, cell yield, phenotype and functionality. An implementation provides for an inoculum expansion and Quantum® system expansion of HSC CB-CD34+ HSCs in co-culture with mesenchymal stem cells (MSCs), an *in situ* source of SDF-1, for about 14 days. Further implementations provide for improving, for example, yield, phenotype and functionality with a shortened monoculture protocol. Human cord blood-derived CD34+ HSCs may be expanded in two phases, for example: (1) Inoculum prep expansion of about 1 million CB-CD34+ HSCs in a T25 flask for about 3 days, followed by (2) the expansion of viable CD34+ HSCs by perfusion in the Quantum® Cell Expansion System for about 5 days to maintain the HSC phenotype of CD34+CD38-CD133+ and related engraftment function by using a monoculture technique with fibronectin-immobilized SDF-1 and other growth factors/cytokines. For example, implementations provide for: (1) the use of a shortened timeline for cell expansion of about 8 days: in flask for about 3 days and in the Quantum® System for about 5 days using, for example, (1) an immobilized SDF-1 signaling factor coupled with (2) a novel growth factor cocktail utilizing, for example, one or more of: SCF, TPO, Flt-3L, IL-3, IL-6, GNDF ± SR-1, and combinations thereof, in monoculture. In an implementation, a monoculture protocol(s), e.g., a shortened monoculture protocol(s), may use a bi-directional cell reseeding task(s) in the Quantum® System, for example.

[0161] CD34+ mixed cell expansion

[0162] *Flask Study*

[0163] In an example, a pilot flask study is conducted over seven days. Cord blood-derived CD34+ HSCs were grown at 37°C with CO_2 CD34 complete medium without shaking. Cells were seeded on day 1 at $1X10^5$ cells/mL in 7 mL and harvested at day 7. A yield of 5,700,000 was considered optimal. CD34 medium produces 11,800,000 cells, while CD34 medium at 1:10 dilution in complete medium produced 9,200,000 cells and CD34 medium at 1:20 dilution produced 5,900,000 cells. Cell viability was 86.2%, 90.3%, and 90.1% for undiluted, 1:10 dilution, and 1:20 dilution CD34 medium, respectively (n=2 per arm, with cells counts performed in triplicate).

[0164] *Freeze-thaw study*

[0165] The feasibility of expanding thawed, mixed cord blood-derived $CD34^+$ HSCs (Stem Cell Technologies, Lot 1907519003, was tested. The cells ($1.1X10^6$ and $2.1x10^6$ CB-derived CD34+ HSCs are seeded in two, separate monoculture Quantum runs, respectively) were cultured in SCGM media (Cat. 20802-0500, CellGenix GmbH, Freiburg, Germany) with a modified supplement cocktail (StemSpan CD34+ supplement at 1% by volume plus GDNF and SR-1) using fibronectin-immobilized SDF-1 coated surfaces in T25 flasks. Both the flasks and the Quantum CESs are coated overnight at 37C with the Fibronetin-SDF-1 protein mixture prior to cell seeding. Fluidics-wise, flasks were in a static condition, whereas the Quantum systems were perfused overnight (12-15 hours) using the Quantum CES “Coat Bioreactor” Task (IC Inlet @ 0 mL/min, IC Circ @ 20 mL/min with Fn/SDF-1, EC Inlet @ 0.1 mL/min with PS, and EC Circ @ 30 mL/min, EC outlet). The cells were cultured for 3 days and in the Quantum® System hollow fiber membrane (HFM) bioreactor for a period of 5 days.

[0166] In the feasibility study, two media formulations with SCF, TPO, Flt-3L, IL-3, IL-6, and GNDF and with or without SR-1 cocktail are evaluated for their ability to support the expansion of CB-derived CD34+ HSCs in monoculture. Both experimental arms are seeded with $1\text{x}10^6$ cells in T25 flasks. On day 3, the Q1893 (without SR-1) Quantum® system and Q1894 (with SR-1) Quantum® system are seeded with cell inoculums from their respective flask cultures.

[0167] On day 8, harvest yields are $4.49\text{x}10^7$ cells without SR-1 (viability 98.5%) and $5.57\text{x}10^7$ (viability 98.8%) cells with SR-1. Flow cytometry analysis of the cryopreserved hematopoietic stem cell Quantum® system harvest phenotype indicated the $CD34^+$ cell fraction was $1.40\text{x}10^7$ cells or 31.1% of the total harvest without SR-1 and $2.1\text{x}10^7$ or 37.7% of the total harvest with SR-1. The minimum and maximum CD34+ doses are 7,000,000 and 10,500,000 cells, respectively.

[0168] Example 2. Monoculture Expansion Strategy for Cord Blood-Derived CD34+ HSCs in the Quantum® System

[0169] An implementation provides an automated expansion protocol for CB-derived CD34+ HSCs in the Quantum® system's dynamic perfusion-based, 2-chambered, semi-permeable hollow fiber membrane (HFM) bioreactor using a novel cytokine cocktail that may be comprised of, for example, SCF, TPO, Flt-3L, IL-3, IL-6, and Fibronectin-SDF-1 coated membrane, and the cocktail can be supplemented with GDNF and SR-1. In addition, the intracapillary (IC) HFM lumen may be coated with a mixture of human fibronectin and the chemokine SDF-1 to mimic the stimulatory and homing effects of bone marrow-derived mesenchymal stromal cells.

[0170] In a series of tests of this automated expansion protocol, three master lots of thawed cord blood (CB) derived, preselected, mixed CD34+ HSCs are expanded in an about 2.1m^2 HFM bioreactor with an about 124 mL IC volume with an initial cell seeding of 2.0×10^6 of the CD34+ HSCs. First, cells are resuspended in SCGM base medium supplemented with the growth factor cocktail. The cells are thawed at 37°C in a water bath, washed in 23 mL of complete medium, and resuspended in 50 mL of complete serum-free GMP SCGM medium (Cat. 20802-0500, CellGenix GmbH, Freiburg, Germany) supplemented with StemSpan CD34 Supplement 10X (Cat. 2691, Stem Cell Technologies, Vancouver, BC, Canada), which contains recombinant human FMS-like tyrosine kinase 3 ligand (Flt3l), stem cell factor (SCF), thrombopoietin (TPO), interleukin 3 (IL-3), and interleukin 6 (IL-6) at a concentration of 1% by volume, Glial cell-derived neurotrophic factor (GDNF) at 10 ng/mL (Cat. 212-GD-050, R&D Systems, Minneapolis, MN, USA), StemRegenin 1 (SR-1) at 0.75 μM (Cat. 72342, Stem Cell Technologies, Vancouver, Canada), and Penicillin-Streptomycin-Neomycin (PSN) antibiotic mixture 100X at 1% by volume (Cat. 15640-055, ThermoFisher Scientific, Waltham, MA, USA).

Base medium may be formulated with serum-free GMP SCGM supplemented with SR-1 and PSN antibiotic mixture.

[0171] Prior to seeding the CD34$^+$ HSC inoculum, the Quantum® System HFM bioreactor (S.A. of 21,000 cm^2) is coated overnight with a mixture of 5 mg of human plasma-derived fibronectin (or 0.23-0.24 µg/cm^2, Cat. 356008, Corning Life Sciences, Corning, NY, USA) and recombinant human Stem Cell Derived Factor 1 (SDF-1) at 0.075 ng/cm^2 (Cat. 6448-SD, R&D Systems, Minneapolis, MN, USA) in 100 mL of PBS w/o Ca^{2+}-Mg^{2+} (Cat. 17-516Q, Lonza Group, Walkersville, MD, USA) at a temperature of 37°C and mixed gas (5%CO_2, 20%O_2, balance N_2).

[0172] The cells were then introduced into the intracapillary loop (e.g., the IC loop) of the HFM bioreactor through a defined perfusion protocol and maintained within the lumen of the bioreactor with a custom counter-flow fluidics program. CB-derived CD34+ HSCs were seeded in suspension into the coated HFM bioreactor in 50 mL of complete medium (serum-free GMP SCGM base medium with the following cytokine cocktail: SCF, TPO, Flt-3L, IL-3, IL-6, GDNF, and SR-1) after lumen and extracapillary medium exchange and conditioning (cell expansion medium is conditioned in the Quantum CES by circulating the medium by perfusion through the IE/EC loops of Quantum system bioreactor for at least 10 minutes using the Quantum embedded task entitled “Condition Media” with the following circulation rates: IC Circ @ 100 mL/min, EC Circ @ 250 mL/min, and EC Inlet @ 0.1 mL/min. This equilibrates the mixed gas (20%O_2, 5%CO_2, and balance N_2) in the bioreactor medium by gas exchange in the EC Loop via gas transfer module), expanded in monoculture, and harvested on day 8 of cell culture using Quantum® System automated tasks, as outlined in the automated task settings shown in following Tables 1-3:

Table 1 (enlarged Part 1 of 3): Quantum CD34+ Cell Seeding Task(s)

Cell Line	PBS		**Cell Line**	None	**Cell Line**	Cells
IC Media Line	N/A		**IC Media Line**	Complete	**IC Media Line**	Complete
EC Media Line	N/A		**EC Media Line**	Base	**EC Media Line**	Base
Reagent Line	N/A	Fn + SDF-1	**Reagent Line**	None	**Reagent Line**	None
Wash Line	N/A		**Wash Line**	None	**Wash Line**	None
Day -1 to 0					**Day 0 to 2**	

		Load Expansion Set	**Prime**	**Coat Bioreactor**	**IC EC Washout**	**Condition Media**	**Load CD34+ Cells** (2-20 x 10^6 cells)		**Condition BR**	**Feed Cells**
		Quantum System Prep					**Custom 1 (Load Cells and Feed for 2 Days)**			
							Step 1	*Step 2*	*Step 3*	*Step 4*
		STEP 1	STEP 2	STEP 3	STEP 4	STEP 5	STEP 5	STEP 6	STEP 7	STEP 8
Task Settings	**IC inlet**	Default settings	Default settings	Default settings	EC media	None	Cell	IC Media	IC Media	IC Media
	IC inlet rate				100	0	50	50	80	0.1
	IC circ rate				-17	20	0	0	-40	-0.1
	EC inlet				EC Media	None	None	None	None	None
	EC inlet rate				148	0	0	0	0	0
	EC circ rate				-1.7	30	30	30	30	30
	Outlet				IC and EC outlet	EC outlet	IC outlet	IC outlet	EC outlet	EC outlet
	Rocker				In Motion (-90°,180°,1sec)	Stationary (0°)	In Motion (-90°,180°,1sec)	In Motion (-90°,180°,1sec)	In Motion (-90°,180°,1sec)	Stationary (0°)
	Stop condition				Exchange (2.5 IC volume; 2.5 EC volume)	Manual (≥ 10 min)	Empty bag	IC volume (50 ml)	IC Volume (310 mL)	Manual (2880 min)

Table 1 (enlarged Part 2 of 3): Quantum CD34+ Cell Seeding Task(s)

		Cell Line	PBS		**Cell Line**	None
		IC Media Line	N/A		**IC Media Line**	Complete
		EC Media Line	N/A		**EC Media Line**	Base
		Reagent Line	N/A	Fn + SDF-1	**Reagent Line**	None
		Wash Line	N/A		**Wash Line**	None
		Day -1 to 0				
		Load Expansion Set	**Prime**	**Coat Bioreactor**	**IC EC Washout**	**Condition Media**
		Quantum System Prep				
		STEP 1	STEP 2	STEP 3	STEP 4	STEP 5
Task Settings	**IC inlet**	Default settings	Default settings	Default settings	EC media	None
	IC inlet rate				100	0
	IC circ rate				-17	20
	EC inlet				EC Media	None
	EC inlet rate				148	0
	EC circ rate				-1.7	30
	Outlet				IC and EC outlet	EC outlet
	Rocker				In Motion (-90°,180°,1sec)	Stationary (0°)
	Stop condition				Exchange (2.5 IC volume; 2.5 EC volume)	Manual (≥ 10 min)

Table 1 (enlarged Part 3 of 3): Quantum CD34+ Cell Seeding Task(s)

Cell Line	Cells		
IC Media Line	Complete		
EC Media Line	Base		
Reagent Line	None		
Wash Line	None		
Day 0 to 2			
Load CD34+ Cells (2-20 x 10^6 cells)		**Condition BR**	**Feed Cells**
Custom 1 (Load Cells and Feed for 2 Days)			
Step 1	*Step 2*	*Step 3*	*Step 4*
STEP 5	STEP 6	STEP 7	STEP 8
Cell	IC Media	IC Media	IC Media
50	50	80	0.1
0	0	-40	-0.1
None	None	None	None
0	0	0	0
30	30	30	30
IC outlet	IC outlet	EC outlet	EC outlet
In Motion (-90°,180°,1sec)	In Motion (-90°,180°,1sec)	In Motion (-90°,180°,1sec)	Stationary (0°)
Empty bag	IC volume (50 ml)	IC Volume (310 mL)	Manual (2880 min)

Table 2: Quantum CD34+ Cell Redistribution and Increase Feeding Task(s)

Cell Line	None
IC Media Line	Complete
EC Media Line	Base
Reagent Line	None
Wash Line	None

		Day 2 to 8		
		CD34+ Cell Redistribution		**Feed Cells**
		Custom 2 (Cell Redistribution and increase Feed)		
		Step 1	*Step 2*	*Step 3*
		STEP 9	STEP 10	STEP 11
Task Settings	**IC inlet**	None	IC Media	IC Media
	IC inlet rate	0	50	0.2*
	IC circ rate	300	-30	-0.1*
	EC inlet	None	None	None
	EC inlet rate	0	0	0
	EC circ rate	100	30	100
	Outlet	EC outlet	EC outlet	EC outlet
	Rocker	In Motion (-90°,180°,1sec)	In Motion (-90°,180°,1sec)	Stationary (0°)
	Stop condition	Time (4 min)	IC Volume (150 mL)	Manual (1440 min)

Table 3: Quantum CD34+ Cell Harvest

		Cell Line	None
		IC Media Line	Complete
		EC Media Line	Base
		Reagent Line	None
		Wash Line	None
		Day 8 – Harvest	
		CD34+ Cell Harvest	
		Custom 3 (Harvest)	
		Step 1	*Step 2*
		STEP 12	STEP 13
Task Settings	**IC inlet**	None	IC media
	IC inlet rate	0	100
	IC circ rate	300	-20
	EC inlet	None	EC Media
	EC inlet rate	0	60
	EC circ rate	300	30
	Outlet	EC outlet	Harvest
	Rocker	In Motion (-90°,180°,1sec)	In Motion (-90°,180°,1sec)
	Stop condition	Time (4 min)	IC Volume (400 mL)

[0173] Default tasks are used for Quantum® System priming, IC media/EC media exchange, and media conditioning tasks. In the process, glucose and lactate levels were monitored by i-STAT Analyzer G and CG4+ cartridges (Abbott Point-of-Care, Princeton, NJ). During cell expansion, the Quantum® System IC and EC inlet flow rates were adjusted in response to the glucose consumption and lactate generation rates and the nature of the automated task. The program uses a gas mixture of about 5%CO_2, about 20%O_2, and balance N_2 at about 37°C for a period of only about 8 days, to reduce (i.e., to minimize) T cell differentiation during cell culture. Cells are harvested using an automated suspension cell protocol.

[0174] For example, Quantum System inlet flow rate(s) may range from about +0.1 to about 100 mL/min, and IC circulation flow rate(s) may range from about -40 to about 300 mL/min. Corresponding Quantum System EC inlet flow rate(s) may range from about 0 to about 148 mL/min, and EC circulation rate(s) may range from about -1.7 mL/min to about 300 mL/min during the cell culture process. During expansion, glucose and/or lactate levels may be analyzed by i-STAT analyzers (e.g., Abbott Point-of-Care, Princeton, NJ, USA) using G and CG4+ cartridges, for example. At harvest, cells were counted (e.g., with a Vi-CELL XR cell analyzer, Beckman Coulter, Indianapolis, IN, USA) (FIG. 17), which included quantification of cell viability by trypan blue (FIG. 18), cryopreserved in CryoStor CS10 freeze medium (e.g., Biolife Solutions, Bothell, WA, USA), and stored in liquid nitrogen vapor phase until further analysis.

[0175] *Expansion results*

[0176] The mean harvest yield, was about 1.02 x 10^8 cells (ranging about 4.02 x 10^7 to about 1.61 x 10^8 cells) with a mean cell viability by trypan blue of about 95.5% (ranging about 93.3% to about 96.8%) and determined by a cell viability counter (Vi-CELL™ XR, Beckman Coulter). The cell expansion yield of 4.0 x 10^7 – 1.6 x 10^8 cells exceeded a minimum CD34$^+$ cell dose of 1.5 x 10^5 cells/kg for a single-unit graft and a minimum CD34$^+$ cell dose of 1.0 x 10^5 cells/kg for a double-unit graft. This equates to minimum doses of 1.1 x 10^7 CD34$^+$ HSCs and 1.4 x 10^7 CD34$^+$ HSCs for a single- and a double-unit graft, respectively, for a 70 kg patient.

[0177] The mean cell population doubling is about 5.4, the mean cell population doubling time is about 34.9 hours and the mean-fold increase may be 51.0-fold (ranging from about 20.1-fold to about 80.5-fold) over the course of the expansion period. IC medium input perfusion flow rates were adjusted in response to glucose and lactate metabolites and range from about 0.1 to about 0.2 mL/min.

[0178] A median cord blood unit (CBU) may contain about 4.4 x 10^6 CD34+ HSCs up to a maximum of about 2.0 x 10^7 CD34$^+$ HSCs. Using the methods and systems described herein the average expansion yields from a single CBU, may be on the order of 2.2 x 10^8 to 1.0 x 10^9 CB-derived stem or progenitor cells, for example, with an automated 8 day monoculture cell expansion protocol by simply increasing the cell inoculum from 2.0 x 10^6 cells up to 4.4 x 10^6 – 2.0 x 10^7 cells with a full CBU CD34$^+$ cell fraction. This approach, among other things, can increase the cell seeding density from, for example, 1.6

x 10^4 cells/mL to 3.6 x 10^4 - 1.6 x 10^5 cells/mL in the perfusion bioreactor, and result in a shorter expansion timeframe that can reduce the potential for cell differentiation.

[0179] *Cryopreservation*

[0180] Comparing such CD34$^+$ HSC harvests to the pre-cryopreservation viability across various UCB donors revealed a relationship between expansion yields and pre-cryopreservation cell viability (FIG. 19). Quantum CES CD34+ cell viability is measured at harvest, by trypan blue dye exclusion using a BC Vi-CELL XR Cell Analyzer. There is a broad range of pre-cryopreservation CD34+ cell viability. In our study, the pre-cryopreservation cell viability ranged from 84% to 98%. Expanding the CB-derived CD34+ HSCs in the Quantum CES generated a mean harvest cell viability of 95%.

[0181] *Glycolytic metabolism*

[0182] Monitoring the glycolytic metabolism shows that the glucose consumption rate may range from 0 to a high of 0.596 on day 5 and the lactate generate rate may range from 0 to a high of 0.650 mmol/day on day 8 (FIG. 20). The difference in peak days for these two metabolites can be attributed to media flow rate adjustments, differential expression of enzymes controlling glycolytic flux, and the demand for central biosynthetic metabolites during cell expansion.

[0183] *Immunophenotyping*

[0184] Thawed cell harvest samples at 1 x 10^6 cells from each of the three (3) automated CB-derived CD34$^+$ cell expansions are resuspended and washed in complete media, centrifuged at 500g for 5 minutes, resuspended in 100 μL of BD Flow Stain Buffer, blocked with 5 μL of human BD Fc for 10 minutes prior to staining with, the following conjugated stains: BD Pharmingen anti-human CD45-APC-H7 (Cat. 560178), anti-human CD34-APC (Cat. 560940), anti-human CD133-PE (Cat. 566593), anti-human CD38-BB515 (Cat. 564499), anti-human CD41a-APC-H7 (Cat. 561422), anti-human CD3-PE (Cat. 555333), anti-human CD19-PE (Cat. 555413), anti-human CD56 (555516), anti-human CD15-BB515 (Cat. 565236), and 7-AAD (Cat. 559925). The ISHAGE-gating guidelines for enumerating CD34+ HSCs by flow cytometry may be consulted for the immunophenotyping of expanded cells and the CD34+ HSC populations may be subordinated to the CD45+ parent cell populations (Cytometry, 34:61-70, 1998). In addition, the CD34$^+$ gating strategy was verified with a CD-Chex CD34 peripheral blood control (Streck, CD-Chex CD34, Level 3). Cell sample data were acquired on a BD FACSCanto II flow cytometer with BD FACSDiva v9.0 software (10,00 events/sample) and subsequently analyzed with FlowJo v10.7 software.

[0185] As shown in Table 1 and FIGS. 21A and 21B, flow cytometry indicate the mean frequency of the CD45+/CD34+ immunophenotype to be 54.3% (range 51.9 to 57.9%) and the mean frequency of the more primitive $CD133^{+}CD38^{-}$ immunophenotype to be 31.8% (range 25.9 to 39.0%) at harvest on Day 8 of automated culture. These results compare favorably with other $CD34^{+}$ HSC 7-day expansion protocols using SR-1 ($CD34^{+}$ HSCs 10-25%) media and 21-day CD34+CD38- expansion protocols using Nicotinamide (CD34+ HSCs 0.2-4.4%) media in UCB-derived cell culture. The mean frequency of the differentiated cell lineages was 0.5% for lymphocytes ($CD3^{+}$, $CD19^{+}$, $CD56^{+}$), 27.7% for neutrophils ($CD15^{+}$), and 26.5% for platelets ($CD41a^{+}$). The fact that biomarkers for both neutrophils and platelets may be present in expanded CB-derived $CD34^{+}$ HSC population can be attributed, in part, to the cytokine composition of the expansion media which contains the interleukins IL-3 and IL-6. Although used to support $CD34^{+}$ cell expansion, both cytokines are also implicated in the development of myeloid cell lineages.

[0186] Table 1. Cell Population Hierarchy & Statistics

Cell Type	**Percentage**
HSCs	56.0
Single Cells	97.0
Live Cells	96.3
CD45+	99.5
CD34+	51.9
$CD133^{hi}CD38^{lo}$	30.6

[0187] *In vitro CB-CD34+ Clonal Differentiation*

[0188] The MethoCult™ $CD34^{+}$ cell differentiation hematopoietic colony-forming-unit (CFU) assay is performed with MethoCult™ H4034 Optimum medium which may be supplemented with rh-cytokines SCF, GM-CSF, IL-3, G-CSF, and EPO (Stem Cell Technologies, Vancouver, BC, Canada). The cells generated hematopoietic progenitor lineages of GEMM, GM, BFU-E CFUs.

[0189] Briefly, Quantum-harvested UCB-derived CD34+ HSCs may be washed, resuspended in IMDM w/ 2%FBS, diluted in methylcellulose-based medium, vortexed, and seeded at 1.1 mL/35 mm well of medium in multi-well plates using seeding densities of 150, 500, and 1,000 cells/well. The CFU plates may be incubated in a static incubator under 37°C, 5% CO_2, humidity conditions for 14 days after which CFUs in each well may be manually counted and scored (n=6) using an Olympus CKX41 inverted microscope at 4x objective magnification with cellSens 2.2 software.

[0190] After 14 days of methylcellulose-based cell culture in MethoCult Optimum H4034 cytokine medium, the CB-derived CD34+ cell differentiated CFUs averaged 56% for the GM, 23% for GEMM and 21% for BFU-E progenitor lineages of the total CFUs across the three expanded CB-derived CD34+ cell lines (see, *e.g.*, FIG. 22 and FIG. 23). These CFU example results are comparable to prior studies with methylcellulose H4034 cytokine differentiation of electroporated, genetically unmodified CB-derived CD34+ HSCs where the majority of the lineages may be GM-CFU (60%) clones followed by BFU-E (36%) and GEMM-CFU (10%) clones and/or where the majority of both the genetically modified and unmodified clones may also be GM-CFU (60%) followed by BFU-E (18-20%) and GEMM-CFU (5%) clones. The differences in the relative distribution of the CFU clones among these studies can be attributed to variations in the donor CBU cell sources, stem cell selection methods, genetic modifications in some instances, and the cytokine cocktail formulations used in the expansion of the CB-derived CD34+ HSCs prior to differentiation. Other small molecule supplements formulated with cytokines beyond SR-1, may include nicotinamide (an SIRT1 histone deacetylase and ribosylase inhibitor), valproic acid (a histone HDAC1 inhibitor), and UM171 (an inhibitor of histone HDAC1 deacetylation and LSD1 demethylation) which may be options for hematopoietic stem cell culture for the purpose of increasing CB-derived CD34+ cell amplification and improving engraftment.

[0191] The MethoCult™ differentiation assay of harvested cells may generate hematopoietic progenitor lineages of GEMM, GM, BFU-E CFUs. These results, taken as a whole, demonstrated that the automated Quantum® system monoculture protocol(s) can support the expansion of preselected CB-derived CD34+ HSCs for both single and double CBU dose equivalency with minimal lymphocyte residual.

[0192] It will be apparent to those skilled in the art that various modifications and variations can be made to the methods and structure of the present invention without departing from its scope. Thus, it should be understood that the present invention is not limited to the specific examples given. Rather, the present invention is intended to cover modifications and variations within the scope of the following claims and their equivalents.

[0193] While example implementations and applications of the present invention have been illustrated and described, it is to be understood that the invention is not limited to the precise configuration and resources described above. Various modifications, changes, and variations apparent to those skilled in the art may be made in the arrangement, operation, and details of the methods and systems of the present invention disclosed herein without departing from the scope of the present invention.

CLAIMS

What is claimed is:

1. A method of expanding cells, comprising:

introducing a first plurality of cells comprising CD34+ Hematopoietic stem cells (HSCs) into hollow fibers of a hollow fiber bioreactor,

wherein the hollow fibers each comprise an interior lumen and an extracapillary side, and

wherein the hollow fibers comprise a coating on at least one of the lumen surface and the extracapillary surface of the hollow fibers, wherein the coating comprises:

stromal cell-derived factor-1 (SDF-1), and

fibronectin or isoforms, or functional equivalents thereof;

exposing the first plurality of cells in the hollow fibers to growth conditions; and,

expanding at least a portion of the first plurality of cells in the hollow fibers of the bioreactor to generate a second plurality of expanded CD34+ HSCs that are expanded at least 50-fold.

2. The method of claim 1, wherein the first plurality of cells is introduced to the plurality of hollow fibers without any prior purification.

3. The method of any one of the preceding claims, wherein the growth conditions comprise exposing the first plurality of cells to one or more growth factors selected from the group consisting of FMS-like Tyrosine Kinase 3 Ligand (Flt-3L), Stem Cell Factor (SCF), thrombopoietin (TPO), glial-derived neurotrophic factor (GDNF), and combinations thereof.

4. The method of any one of the preceding claims, wherein exposing cells in the hollow fibers to growth conditions comprises circulating a cell growth media through the lumen of the hollow fibers.

5. The method of any one of the preceding claims, wherein exposing cells in the hollow fibers to growth conditions comprises circulating a cell growth media through the extracapillary side of the hollow fibers.

6. The method of any one of the preceding claims, wherein introducing cells into the hollow fibers comprises:

circulating, with a pump, a plurality of cells within the lumen of the hollow fibers;

stopping the pump to allow a portion of the plurality of cells to attach to a first portion of the lumen of the hollow fibers;

rotating the hollow fiber bioreactor 180 degrees from an initial position;

circulating, with the pump, a plurality of cells within the lumen of the hollow fibers; and

stopping the pump to allow a portion of the plurality of cells to attach to a second portion of the lumen of the hollow fibers.

7. The method of any one of the preceding claims, wherein the plurality of cells is introduced simultaneously at both ends of the hollow fibers.

8. The method of any one of the preceding claims, wherein the coating on the at least one of the lumen surface and the extracapillary surface of the hollow fibers, further comprises a material that promotes cellular adhesion to a surface of the hollow fibers.

9. The method of any one of the preceding claims, wherein the coating on the at least one of the lumen surface and the extracapillary surface of the hollow fibers, further comprises a protein moiety.

10. The method of claim 9, wherein the protein moiety is interleukin-21 (IL-21).

11. The method of any one of the preceding claims, wherein expanding cells in the hollow fibers comprises contacting the CD34+ HSCs in the hollow fibers with a media comprising GDNF and an aryl hydrocarbon receptor antagonist.

12. The method of claim 11, wherein the aryl hydrocarbon receptor antagonist is at least one of StemRegenin 1 (SR1) and UM171.

13. The method of claims 11 or 12, wherein the media further comprises at least one of SCF, TPO, Flt-3L, IL-3, IL-6, SDF-1, and fibronectin.

14. The method of any one of claims 11-13, wherein the GDNF is present in the media at a concentration of 0.5% to 2% weight per volume.

15. The method of any one of claims 11-13, wherein the GDNF is present in the media at a concentration of at least 10 ng/mL.

16. The method of any one of the preceding claims, wherein the hollow fibers comprise a first media in the lumen and a second media in contact with the extracapillary side of the hollow fibers.

17. The method of claim 16, wherein the media in the lumen is concentrated in at least one component relative to the concentration of the same component on the extracapillary side of the hollow fibers.

18. The method of claim 17, wherein the component is GDNF.

19. The method of claim 17, wherein the component is selected from the group consisting of SR-1, SCF, TPO, Flt-3L, IL-3, IL-6, SDF-1, and fibronectin.

20. The method of any one of claims 17-19, wherein the at least one component is concentrated at least five-fold.

21. The method of any one of claims 17-19, wherein the at least one component is concentrated at least ten-fold.

22. The method of any one of the preceding claims, wherein the CD34+ HSCs in the hollow fibers are expanded in monoculture, and no additional cell type is co-cultured with the CD34+ HSCs in the hollow fibers.

23. The method of claim 22, wherein the hollow fibers comprise a coating comprising SDF-1 and fibronectin on at least one of the lumen surface and the extracapillary surface of the hollow fibers.

24. The method of any one of claims 1-21, wherein prior to introducing the first plurality of cells into the hollow fibers, mesenchymal stem cells are introduced into the hollow fibers;

exposing the mesenchymal stem cells in the hollow fibers to growth conditions;

thereafter introducing the plurality of cells comprising CD34+ HSCs into the plurality of hollow fibers to co-culture the cells comprising CD34+ HSCs with the mesenchymal stem cells in the hollow fibers.

25. The method of any one of claims 1-21, wherein prior to introducing cells into the hollow fibers, growing a first plurality of mesenchymal stem cells in a static growth chamber;

growing CD34+ HSCs in the static growth chamber in co-culture with the mesenchymal stem cells;

removing a plurality of cells comprising CD34+ HSCs from the static growth chamber; and,

introducing the plurality of cells comprising CD34+ HSCs from the static growth chamber into the hollow fibers.

26. The method of any one of the preceding claims, wherein the plurality of cells comprising CD34+ HSCs are obtained from at least one of cord blood, bone marrow, and peripheral blood.

27. The method of any one of the preceding claims, wherein the CD34+ HSCs that are expanded are CD45+/CD34+ HSCs.

28. The method of any one of the preceding claims, wherein the CD34+ HSCs that are expanded are CD133+CD38- progenitor cells.

29. The method of any one of the preceding claims, wherein the first plurality of cells comprising CD34+ HSCs are from a single unit of blood or tissue and the expanded CD34+ HSCs are sufficient for at least one engraftment procedure of a human recipient.

30. The method of claim 29, wherein the single unit of blood is cord blood.

31. The method of claim 30, wherein the single unit of tissue is bone marrow.

32. The method of any one of the preceding claims, wherein CD34+ HSCs have at least 90% viability after expansion.

33. The method of any one of the preceding claims, wherein the lumen of the hollow fibers is coated with a glycoprotein.

34. The method of any one of the preceding claims, further comprising removing at least a portion of the second plurality of expanded cells from the hollow fibers.

35. The method of claim 34, further comprising administering the second plurality of expanded cells removed from the hollow fibers to a patient.

36. The method of claim 35, wherein the administering reconstitutes hematopoiesis in the patient.

37. A method of capturing cells, the method comprising:

introducing a mixture of target cells and non-target cells into hollow fibers of a hollow fiber bioreactor, wherein the hollow fibers each comprise an interior lumen and an extracapillary side,

wherein the hollow fibers comprise a coating on at least one of the lumen surface and the extracapillary surface of the hollow fibers, wherein the coating comprises:

stromal cell-derived factor-1 (SDF-1), and

fibronectin or isoforms, or functional equivalents thereof;

exposing the mixture to capture conditions;

capturing at least a portion of the target cells on at least one of the lumen and the extracapillary surface of the hollow fibers; and,

flushing at least a portion of the non-target cells from the hollow fibers.

38. The method of claim 37, wherein the coating further comprises a protein moiety selected from the group consisting of interleukin-21 (IL-21), streptavidin, avidin, and anti-biotin antibodies or functional fragments thereof.

39. The method of claims 37 or 38, further comprising, after flushing at least a portion of the non-target cells from the hollow fibers, exposing the portion of the target cells captured to growth conditions; and,

expanding the portion of the target cells captured in the hollow fibers to generate a plurality of expanded target cells.

40. The method of any one of claims 37-39, further comprising, after expanding the portion of the target cells captured in the hollow fibers, removing at least a portion of the plurality of expanded target cells from the hollow fibers.

41. A coated hollow fiber membrane, comprising:

a hollow fiber membrane having a lumen surface and an extracapillary surface;

wherein the hollow fibers comprise a coating on at least one of the lumen surface and the extracapillary surface of the hollow fibers, wherein the coating comprises:

stromal cell-derived factor-1 (SDF-1), and

fibronectin or isoforms, or functional equivalents thereof.

42. The coated hollow fiber membrane of claim 41, wherein the coating further comprises a protein moiety selected from the group consisting of a cytokine, an aptamer, a chemokine, a monoclonal antibody, streptavidin, avidin, a biotinylated molecule, and an anti-biotin antibody or a functional fragment thereof.

43. The coated hollow fiber membrane of claim 42, wherein the chemokine is stromal cell-derived factor-1 (SDF-1) or interleukin-21 (IL-21).

44. The coated hollow fiber membrane of any one of claims 41-43, wherein the membrane comprises at least one of polysulfone or polyethersulfone.

45. A method of forming a coated hollow fiber membrane, comprising:

providing a hollow fiber membrane having a lumen surface and an extracapillary surface;

applying a first coating onto the lumen surface of the hollow fiber membrane, wherein the first coating comprises a material that promotes cellular adhesion to at least one of the lumen of the hollow fiber membrane and the extracapillary surface of the hollow fiber membrane; and

applying a second coating onto the lumen surface of the hollow fiber membrane, wherein the second coating comprises a soluble protein moiety.

46. The method of claim 45, wherein applying the first coating and the second coating material comprises:

conjugating first coating material and the second coating material into a conjugate apart from the hollow fiber membrane; and,

coating the conjugate onto the lumen surface of the hollow fiber membrane.

47. The method of claims 45 or 46, wherein the first coating material is fibronectin, or isoforms, or functional equivalents thereof.

48. The method of any one of claims 45-47, wherein the second coating material comprises at least one of a cytokine, an aptamer, a chemokine, a monoclonal antibody, streptavidin, avidin, a biotinylated molecule, and an anti-biotin antibody or a functional fragment thereof.

49. The method of any one of claims 45-48, wherein the first coating is fibronectin, and wherein the second coating comprises stromal cell-derived factor-1 (SDF-1) or interleukin-21 (IL-21).

50. A composition for expanding CD34+ HSCs, comprising glial cell-derived neurotrophic factor (GDNF) and an aryl hydrocarbon receptor (AHR) antagonist.

51. The composition of claim 50, further comprising at least one of SCF, TPO, Flt-3L, IL-3, IL-6, SDF-1, and fibronectin.

52. The composition of claim 50 or 51, wherein the AHR antagonist is at least one of SR-1 and UM171.

53. The composition of any one of claims 50-52, wherein the GDNF is present at a concentration of 0.5% to 2% weight per volume.

54. The composition of any one of claim 50-53, wherein the GDNF is present at a concentration of at least 10 ng/mL.

55. The composition of any one of claims 50-54, wherein fibronectin and SDF-1 are immobilized on a cell culture surface.

56. The composition of claim 55, wherein the cell culture surface is a semi-permeable membrane.

57. The composition of any one of claims 50-56, wherein the composition increases levels of BCL2 and inhibits HSC differentiation.

58. A method of expanding cells by perfusion in a cell expansion system, the method comprising:

coating a hollow fiber bioreactor with a first fluid, wherein the first fluid comprises a signaling factor and/or a coating factor;

introducing a plurality of cells into the hollow fiber bioreactor, wherein the hollow fiber bioreactor comprises a hollow fiber membrane;

exposing the plurality of cells to a second fluid, wherein the second fluid comprises a plurality of growth factors; and

growing the plurality of cells in the hollow fiber bioreactor in monoculture or in coculture.

59. The method of claim 58, wherein the first fluid is comprises at least one of fibronectin and SDF-1.

60. The method of claim 59, wherein the fibronectin and the SDF-1 are mixed together prior to coating the hollow fiber bioreactor.

61. The method of claim 59, wherein the hollow fiber bioreactor is coated sequentially by coating the hollow fiber bioreactor with the fibronectin and then coating the hollow fiber bioreactor with the SDF-1.

62. The method of claim 59, wherein the hollow fiber bioreactor is coated sequentially by coating the hollow fiber bioreactor with the SDF-1 and then coating the hollow fiber bioreactor with the fibronectin.

63. The method of any one of claims 59 to 62, wherein an amount of the fibronectin used to coat the hollow fiber bioreactor is 0.001 $\mu g/cm^2$ to 2 $\mu g/cm^2$.

64. The method of any one of claims 59 to 62, wherein an amount of the fibronectin used to coat the hollow fiber bioreactor is 0.01 $\mu g/cm^2$ to 1.0 $\mu g/cm^2$.

65. The method of any one of claims 59 to 62, wherein an amount of the fibronectin used to coat the hollow fiber bioreactor is 0.10 $\mu g/cm^2$ to 0.50 $\mu g/cm^2$.

66. The method of any one of claims 59 to 62, wherein an amount of the fibronectin used to coat the hollow fiber bioreactor is 0.20 $\mu g/cm^2$ to 0.40 $\mu g/cm^2$.

67. The method of any one of claims 59 to 62, wherein an amount of the fibronectin used to coat the hollow fiber bioreactor is 0.23 $\mu g/cm^2$ to 0.24 $\mu g/cm^2$.

68. The method of any one of claims 59 to 67, wherein an amount of the SDF-1 used to coat the hollow fiber bioreactor is 0.001 ng/cm^2 to 0.30 ng/cm^2.

69. The method of any one of claims 59 to 67, wherein an amount of the SDF-1 used to coat the hollow fiber bioreactor is 0.01 ng/cm^2 to 0.10 ng/cm^2.

70. The method of any one of claims 59 to 67, wherein an amount of the SDF-1 used to coat the hollow fiber bioreactor is 0.05 ng/cm^2 to 0.09 ng/cm^2.

71. The method of any one of claims 59 to 67, wherein an amount of the SDF-1 used to coat the hollow fiber bioreactor is 0.075ng/cm^2.

72. The method of any one of claims 59 to 71, wherein the second fluid comprises GDNF.

73. The method of claim 72, wherein an amount of the GDNF in the second fluid is 0.001 ng/mL to 40.0 ng/mL.

74. The method of claim 72, wherein an amount of the GDNF in the second fluid is 0.01 ng/mL to 20 ng/mL.

75. The method of claim 72, wherein an amount of the GDNF in the second fluid is 0.10 ng/mL to 15 ng/mL.

76. The method of claim 72, wherein an amount of the GDNF in the second fluid is 1.0 ng/mL to 15 ng/mL.

77. The method of claim 72, wherein an amount of the GDNF in the second fluid is 5.0 ng/mL to 15 ng/mL.

78. The method of claim 72, wherein an amount of the GDNF in the second fluid is 10 ng/mL.

79. The method of any of claims 58 to 78, wherein the plurality of growth factors comprises at least one of SCF, TPO, Flt-3L, IL-3, and IL-6.

80. The method of any of claims 58 to 78, wherein the second fluid comprises StemRegenin (SR-1).

81. The method of claim 80, wherein an amount of the SR-1 in the second fluid is 0.001 μM to 3.0 μM.

82. The method of claim 80, wherein an amount of the SR-1 in the second fluid is 0.01 μM to 2.0 μM.

83. The method of claim 80, wherein an amount of the SR-1 in the second fluid is 0.10 μM to 1.0 μM.

84. The method of claim 80, wherein an amount of the SR-1 in the second fluid is 0.75 μM.

85. The method of any of claims 58 to 84, wherein prior to introducing the plurality of cells into the hollow fiber bioreactor, the method comprises:

coating the hollow fiber bioreactor for a predetermined time period with a mixture of 5 mg of human plasma-derived fibronectin or 0.23-0.24 μg/cm^2 of fibronectin and recombinant human Stem Cell Derived Factor 1 (SDF-1) at 0.075 ng/cm^2.

86. The method of claim 85, wherein the predetermined time period is 4.0 hours to 16.0 hours.

87. The method of claim 85, wherein the predetermined time period is 8.0 hours to 12.0 hours.

88. A cell expansion system comprising the hollow fiber bioreactor that is configured to perform the method of any of claims 58 to 87.

89. The cell expansion system of claim 87, further comprising:

a processor; and

a memory, in communication with and readable by the processor, and containing a series of instructions that, when executed by the processor, cause the processor to perform the method of any of claims 58 to 88.

90. A method of capturing a target species, the method comprising:

introducing a mixture of target species and non-target species into hollow fibers, wherein the hollow fibers each comprise an interior lumen and an extracapillary side,

wherein the hollow fibers comprise a coating on at least one of the lumen surface and the extracapillary surface of the hollow fibers, wherein the coating comprises at least one of streptavidin, avidin, a biotinylated molecule, and an anti-biotin antibody or a functional fragment thereof.

exposing the mixture to capture conditions;

capturing at least a portion of the target species on at least one of the lumen and the extracapillary surface of the hollow fibers; and,

flushing at least a portion of the non-target species from the hollow fibers.

91. The method of claim 90, wherein the target species comprise biotinylated aptamers or biotinylated antibodies that bind to the coating on the at least one of the lumen surface and the extracapillary surface of the hollow fibers.

92. The method of claims 90 or 91, wherein the target species is a cell and further comprising, after flushing at least a portion of the non-target species from the hollow fibers, exposing the portion of the cell captured to growth conditions; and,

expanding the portion of the cell captured in the hollow fibers to generate a plurality of expanded target cells.

93. The method of claim 92, further comprising, after expanding the portion of the target cells captured in the hollow fibers, removing at least a portion of the plurality of expanded target cells from the hollow fibers.

SHEET 1/21

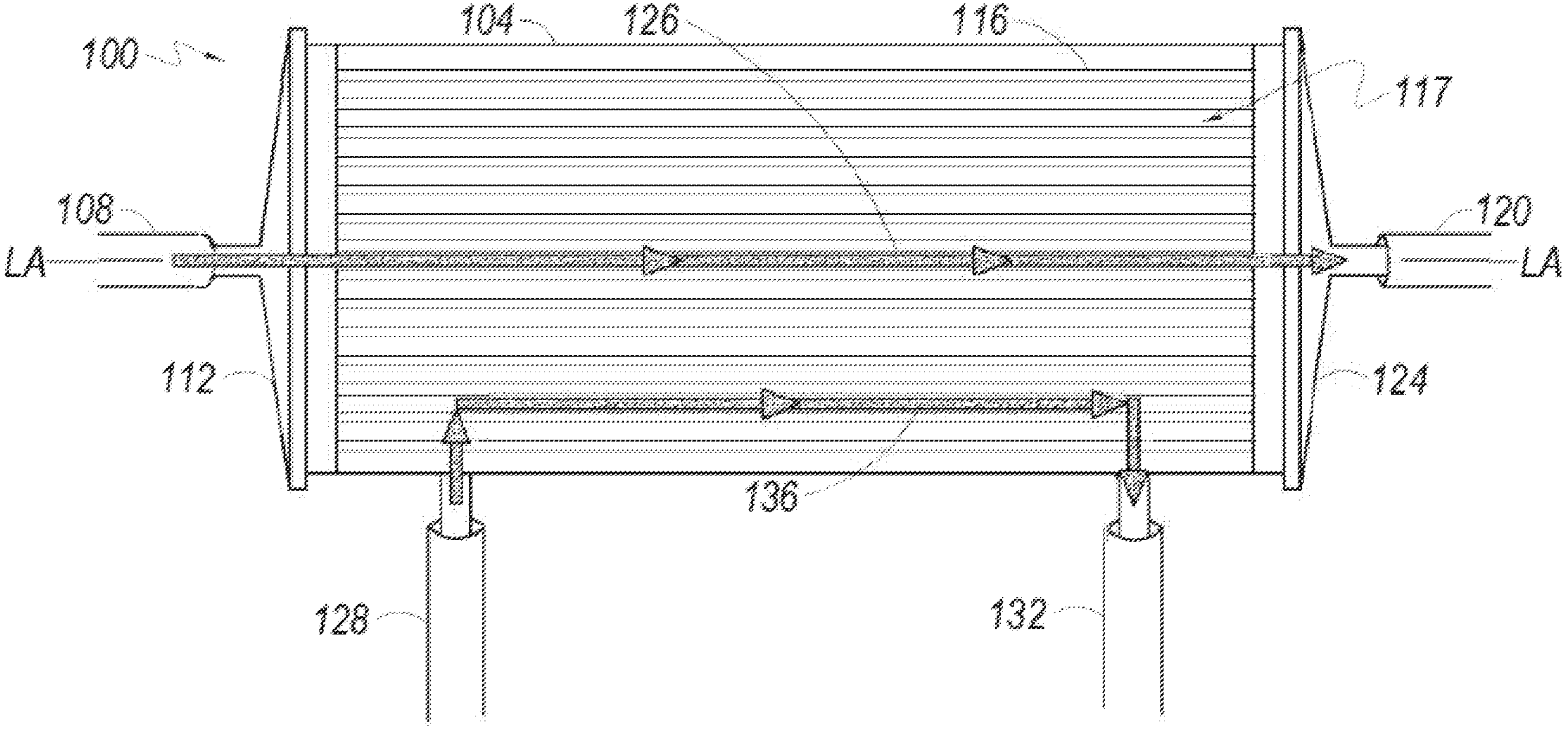

FIG. 1

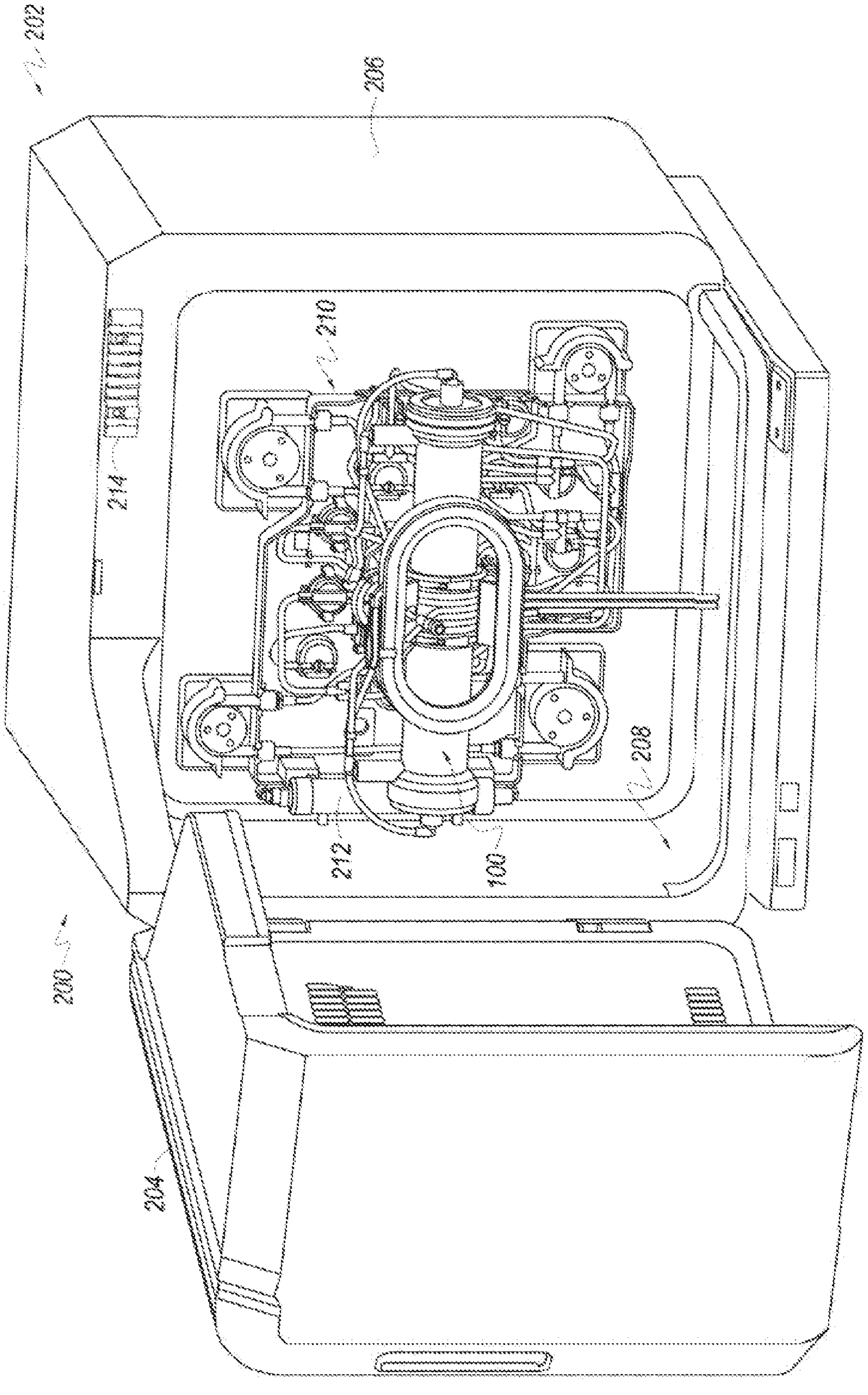

FIG. 2

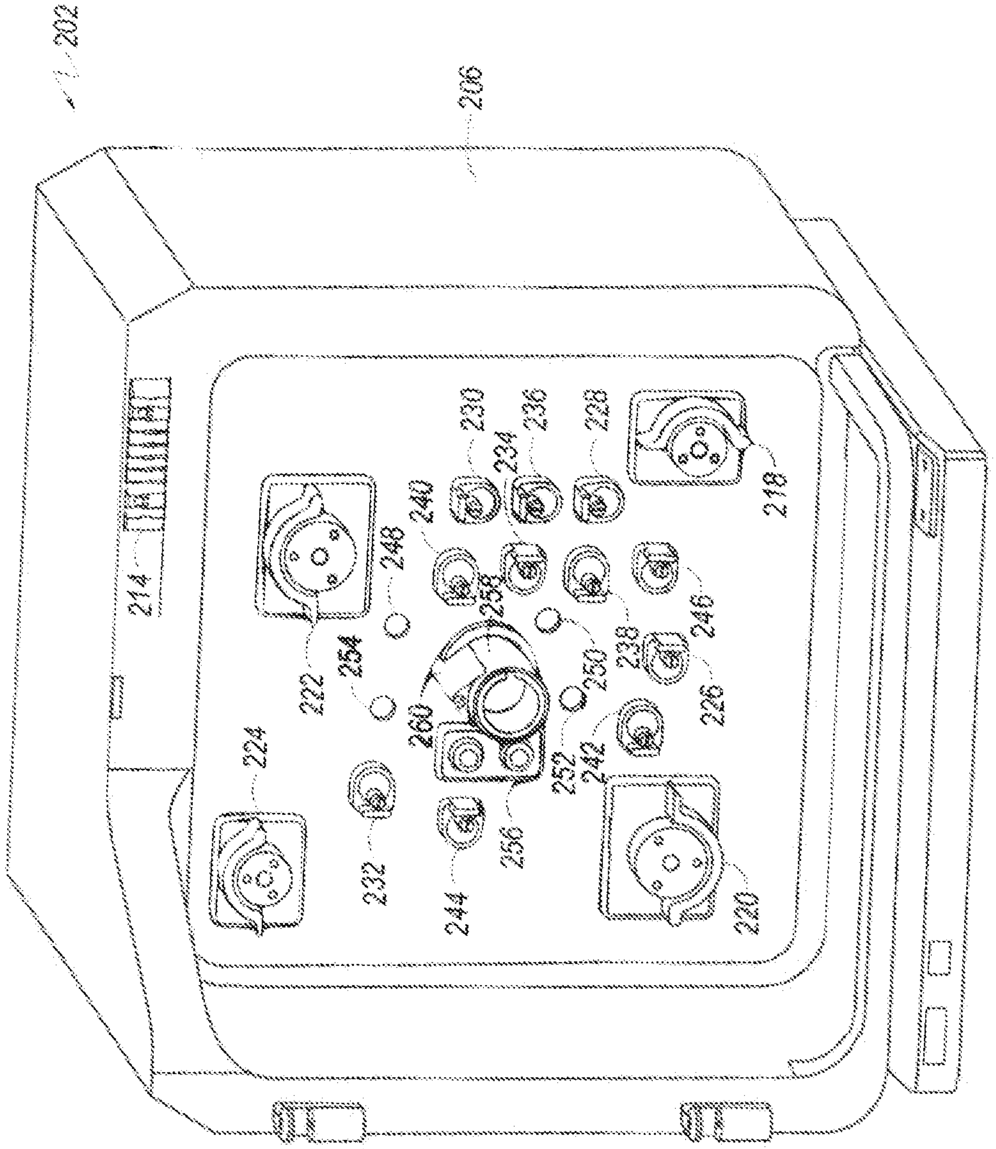
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FIG. 3

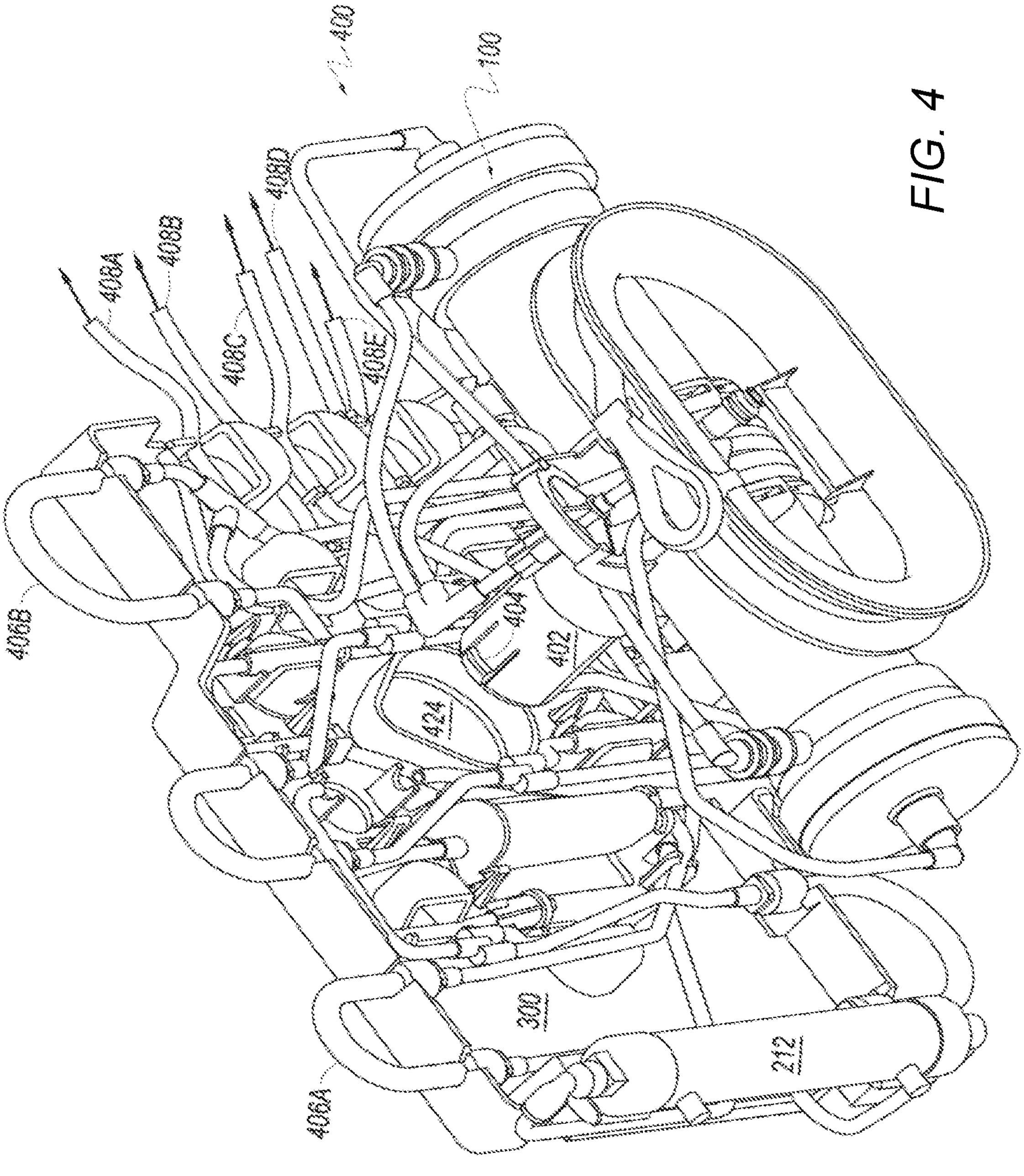

FIG. 4

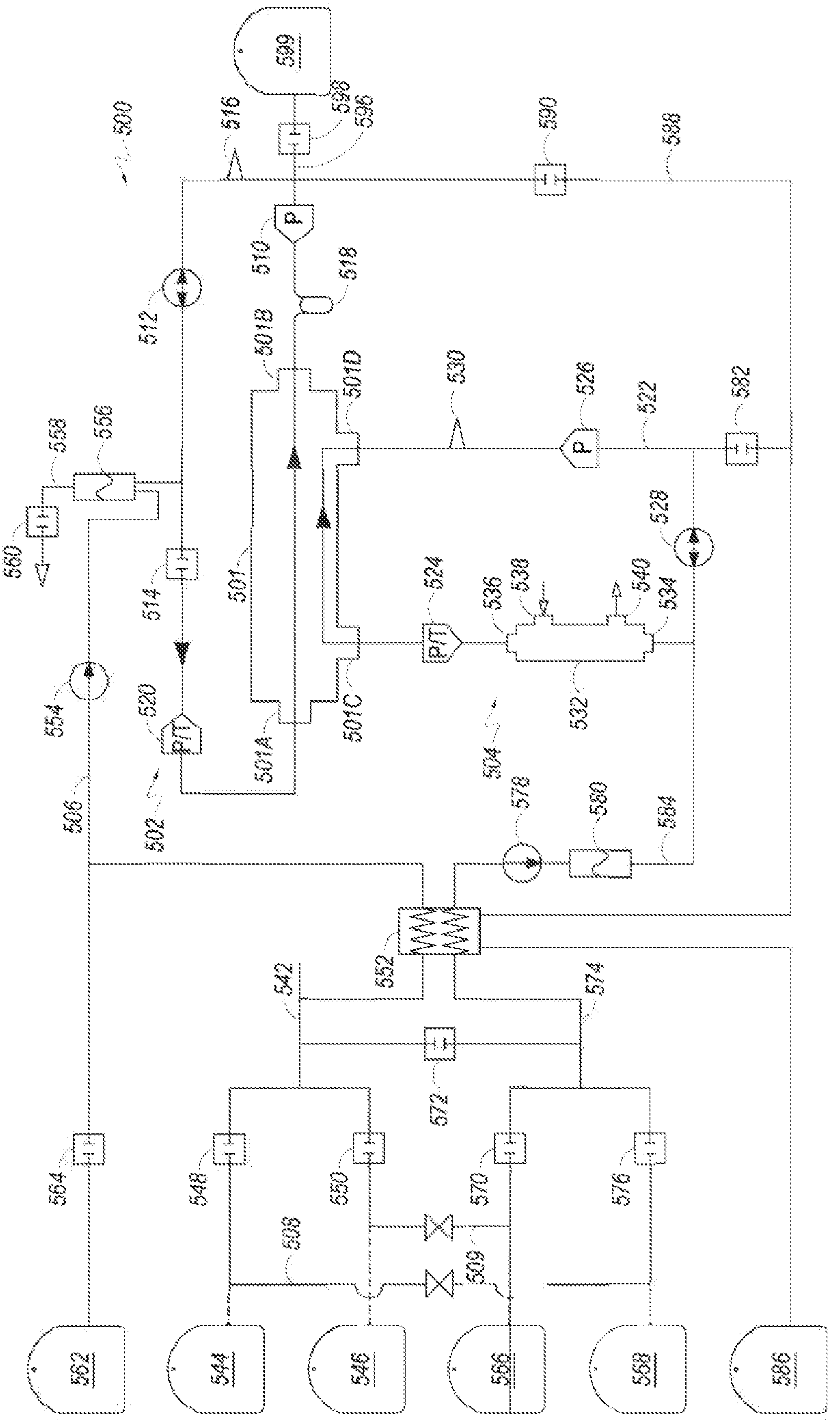
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FIG. 5

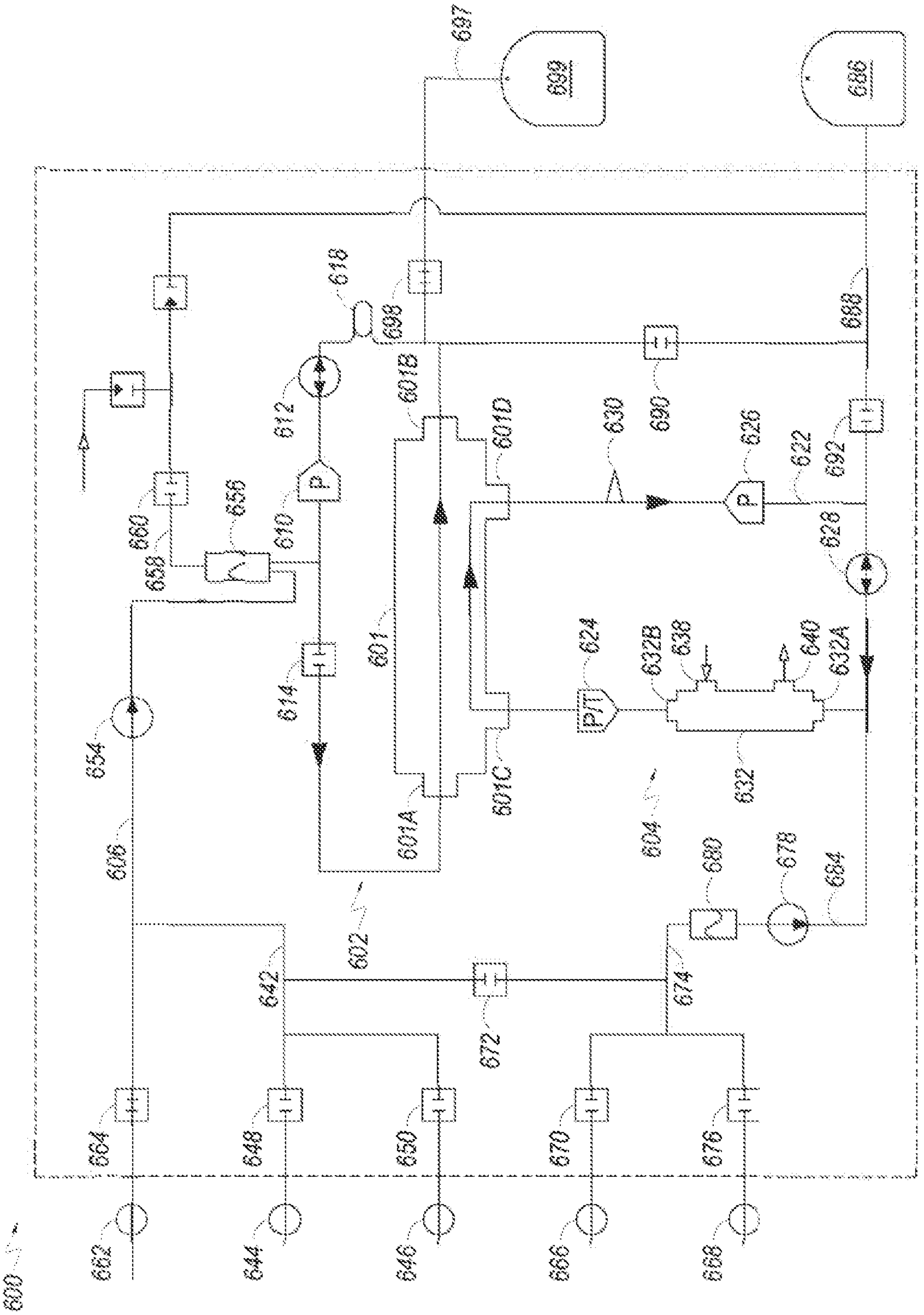
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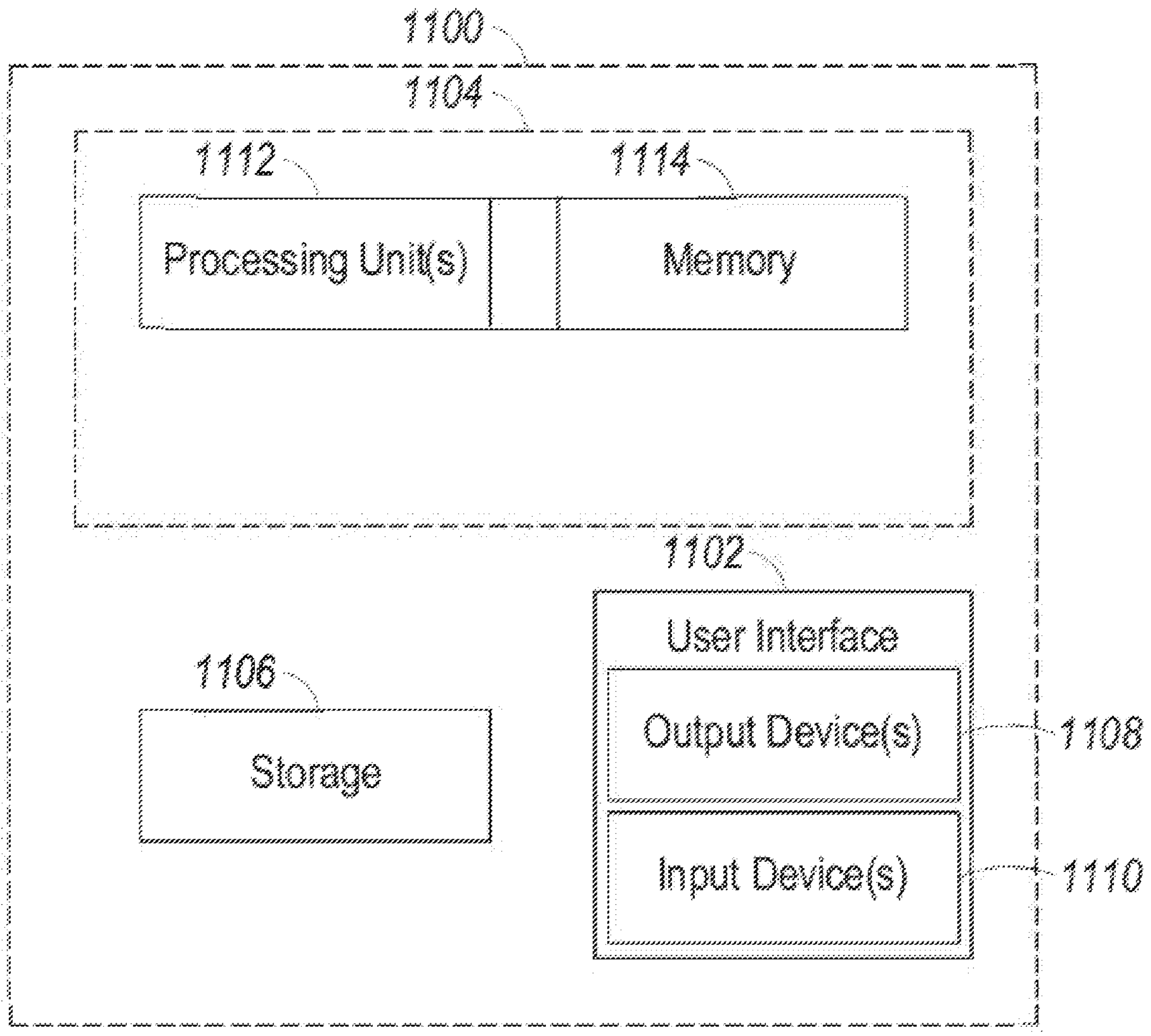
FIG. 6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Memory
1102
User Interface
1106
Storage
Output Device(s)
1108
Input Device(s)
1110

FIG. 7

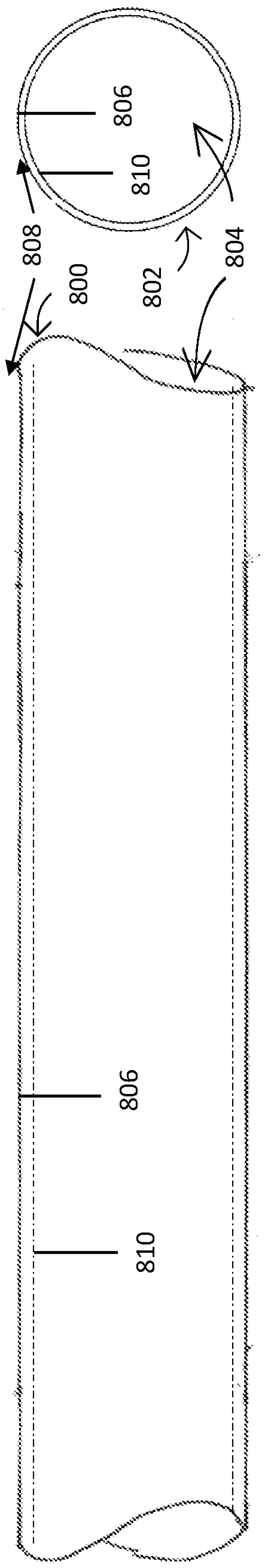


FIG. 8

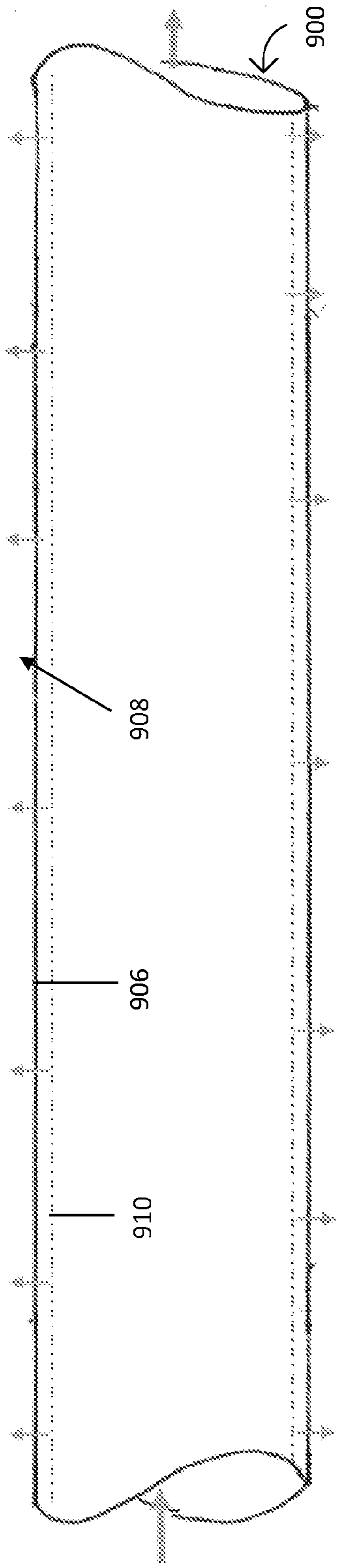


FIG. 9

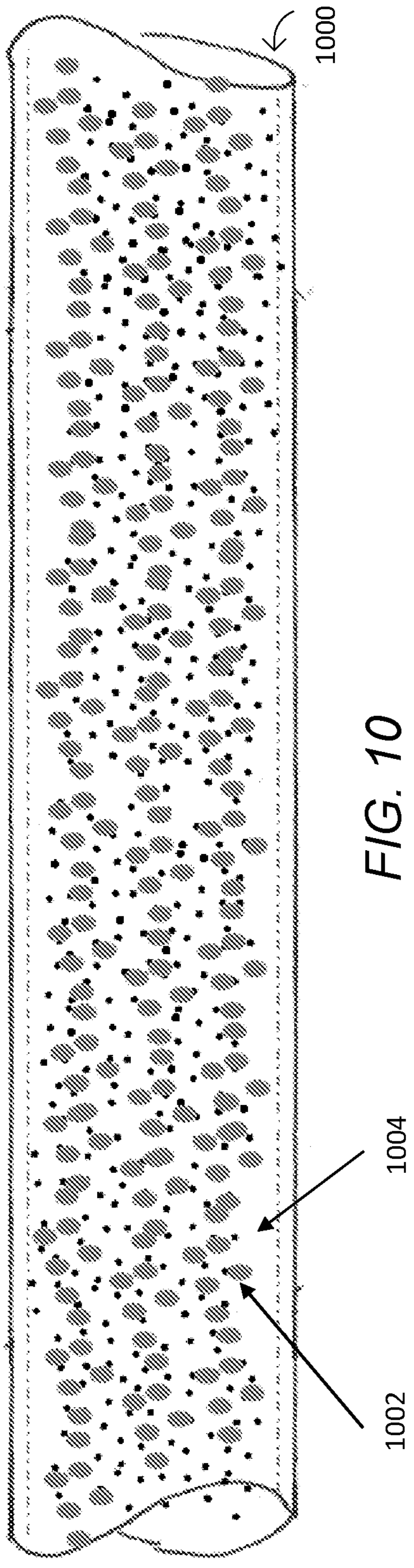


FIG. 10

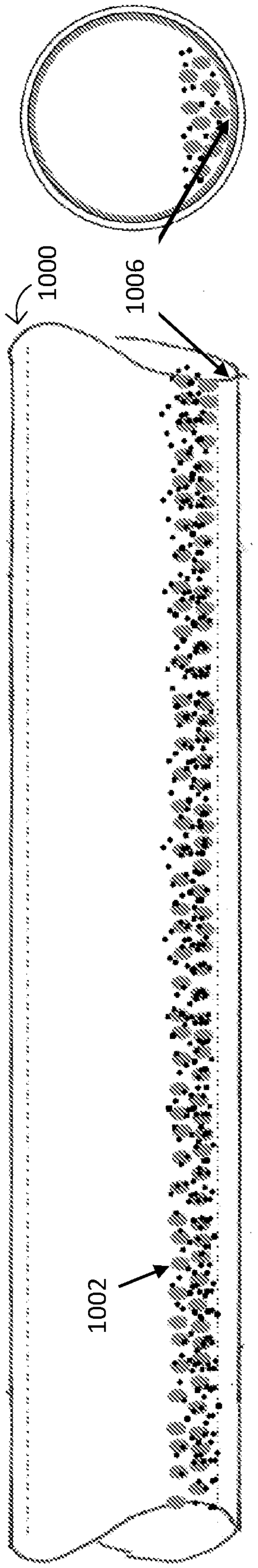


FIG. 11

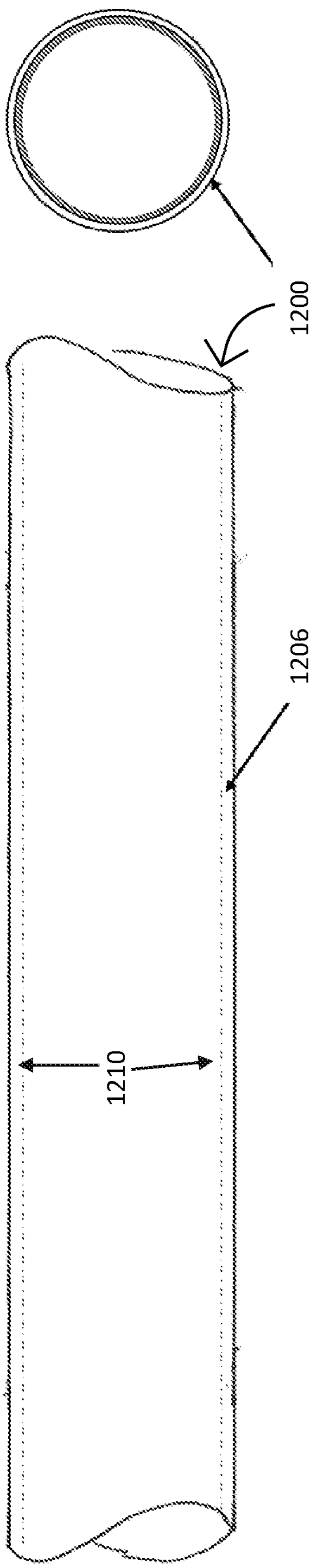


FIG. 12

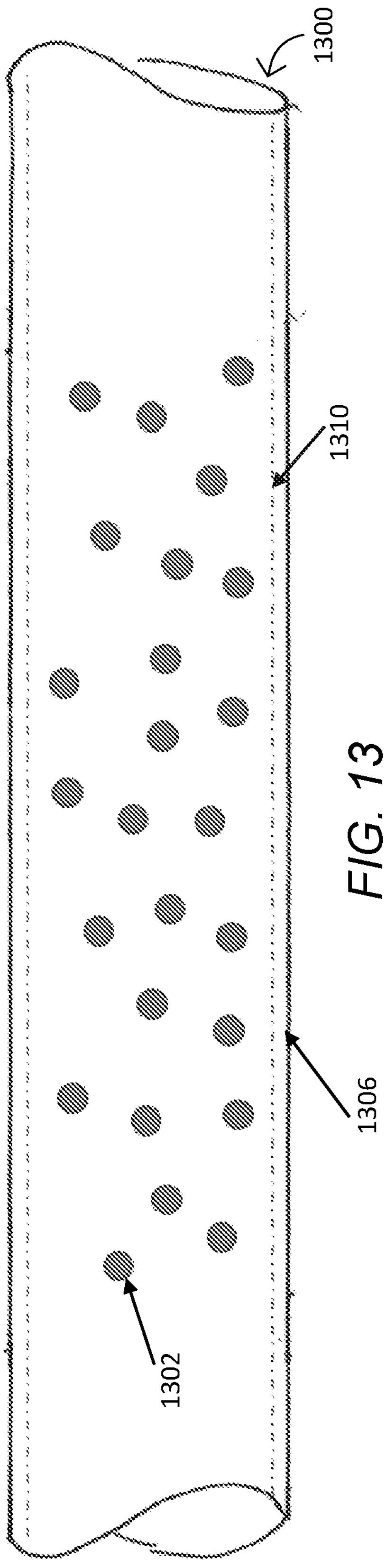
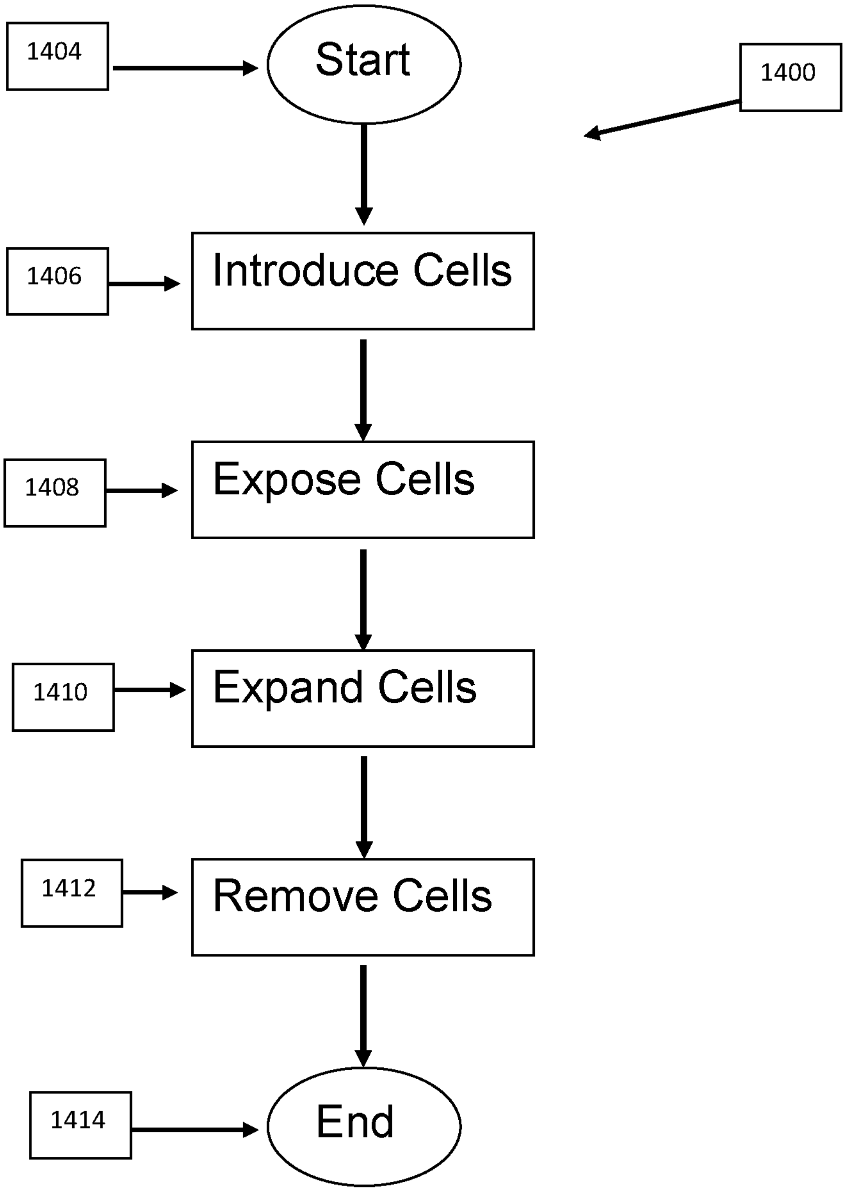


FIG. 13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Introduce Cells
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Expose Cells
1410
Expand Cells
1412
Remove Cells
1414
End

FIG. 14

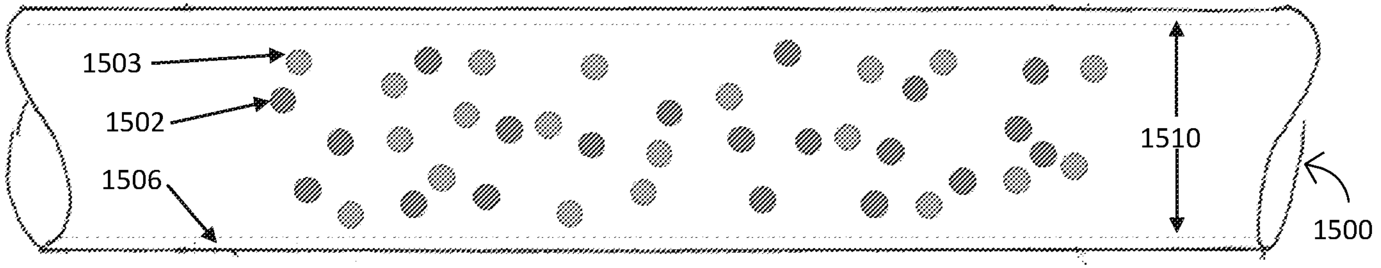


FIG. 15

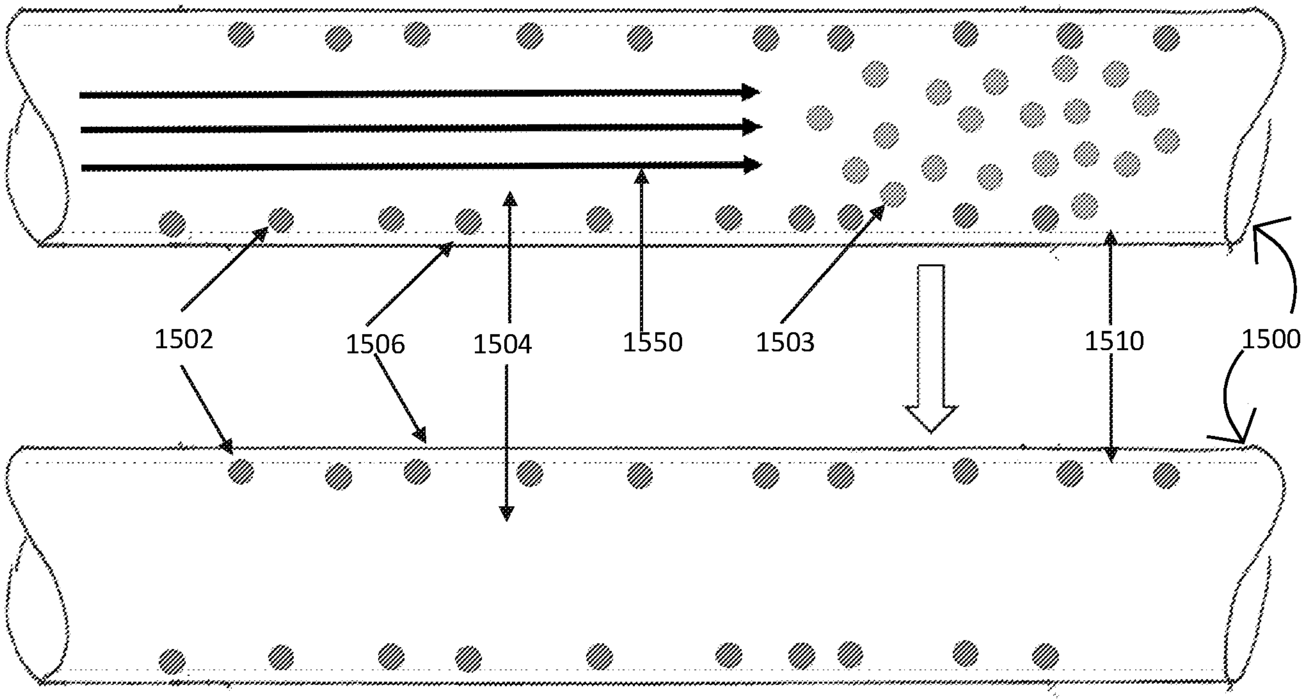


FIG. 16

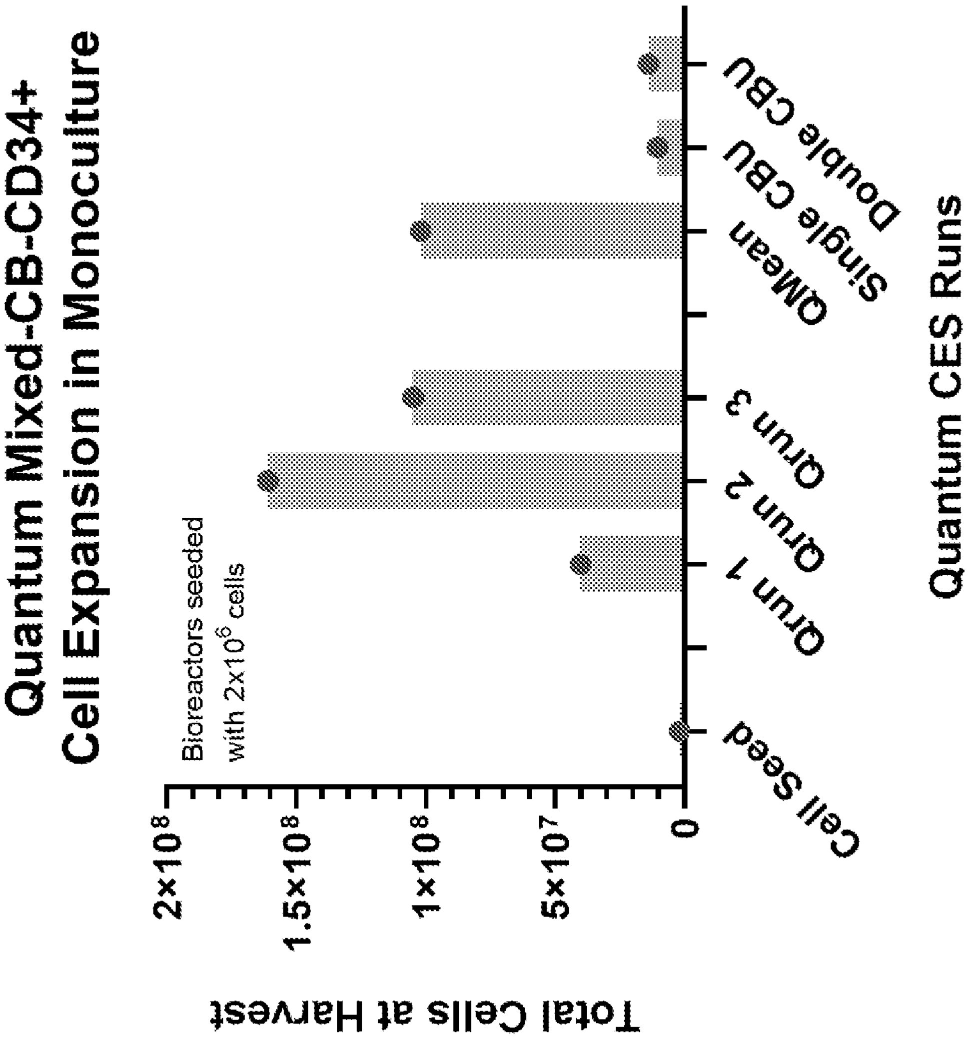

Quantum Mixed-CB-CD34+
Cell Expansion in Monoculture
Bioreactors seeded
with 2x10^6 cells
Total Cells at Harvest
2×10^8
1.5×10^8
1×10^8
5×10^7
0
Cell Seed
Qrun 1
Qrun 2
Qrun 3
QMean
Single CBU
Double CBU
Quantum CES Runs

FIG. 17

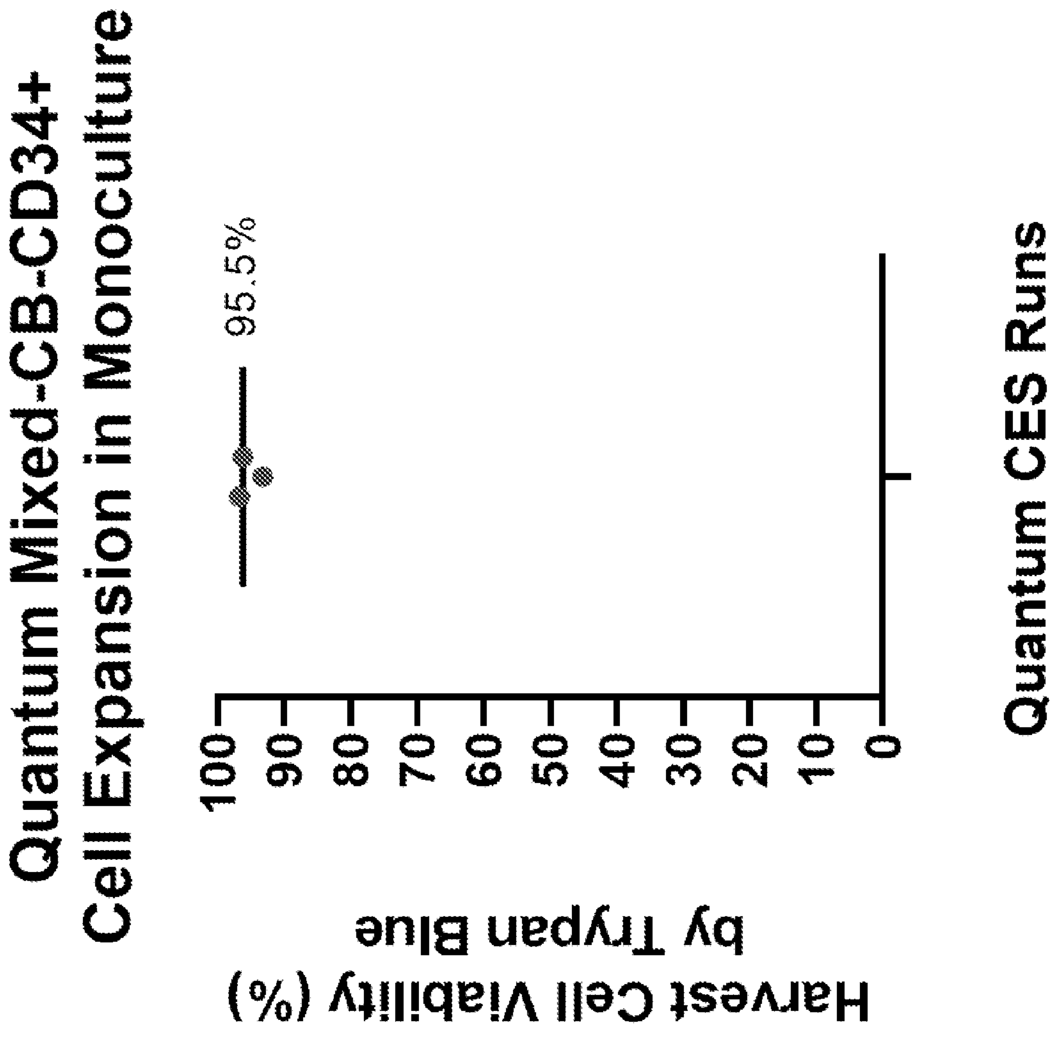

Quantum Mixed-CB-CD34+
Cell Expansion in Monoculture
Harvest Cell Viability (%)
by Trypan Blue
100
90
80
70
60
50
40
30
20
10
0
95.5%
Quantum CES Runs

FIG. 18

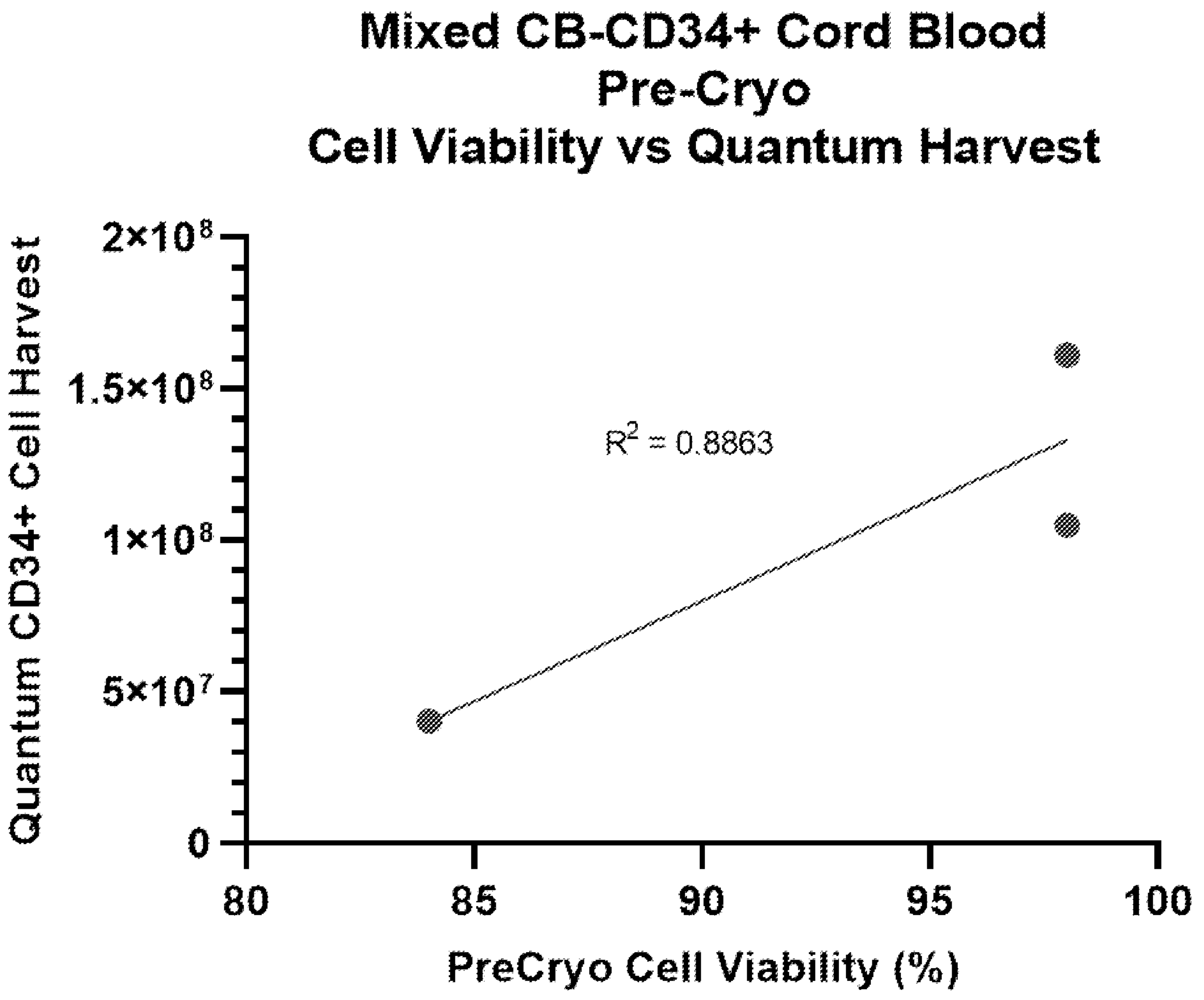

Mixed CB-CD34+ Cord Blood
Pre-Cryo
Cell Viability vs Quantum Harvest
Quantum CD34+ Cell Harvest
2×10⁸
1.5×10⁸
1×10⁸
5×10⁷
0
R² = 0.8863
80
85
90
95
100
PreCryo Cell Viability (%)

FIG. 19

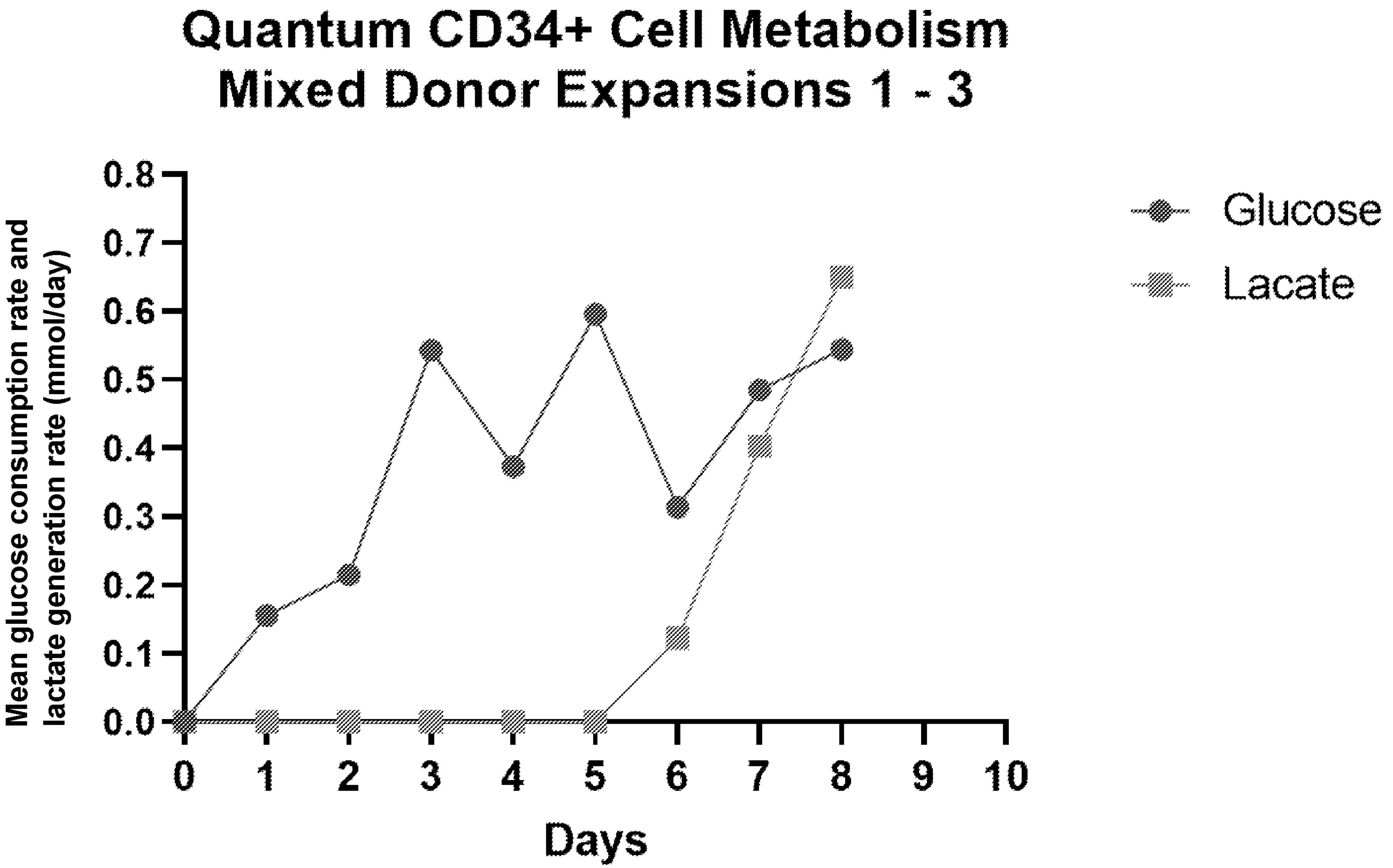

Quantum CD34+ Cell Metabolism Mixed Donor Expansions 1 - 3

FIG. 20

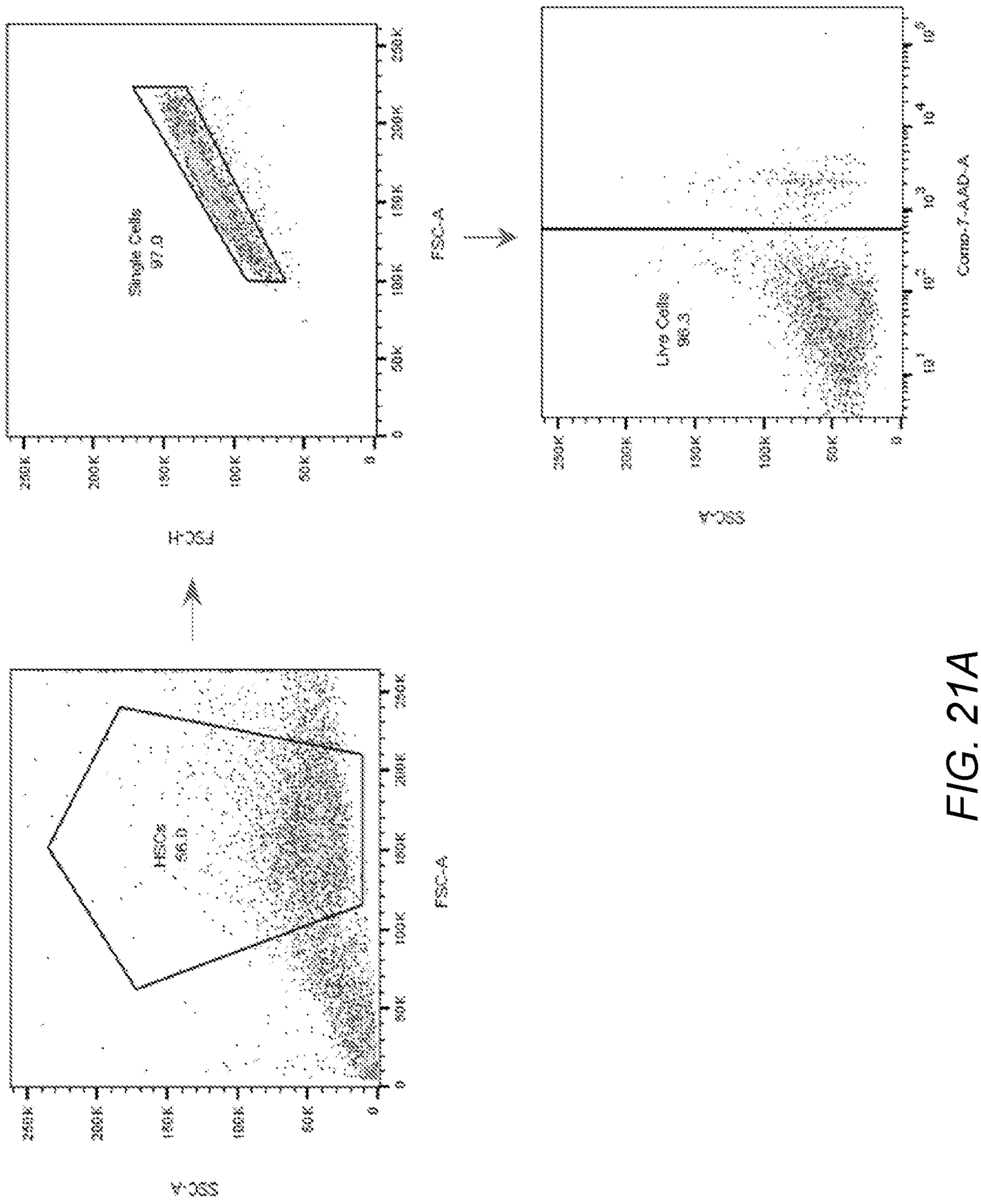

HSCs
56.0
SSC-A
FSC-A
Single Cells
97.0
FSC-H
FSC-A
Live Cells
98.3
SSC-A
Comp-7-AAD-A
0
50K
100K
150K
200K
250K
10^1
10^2
10^3
10^4
10^5

FIG. 21A

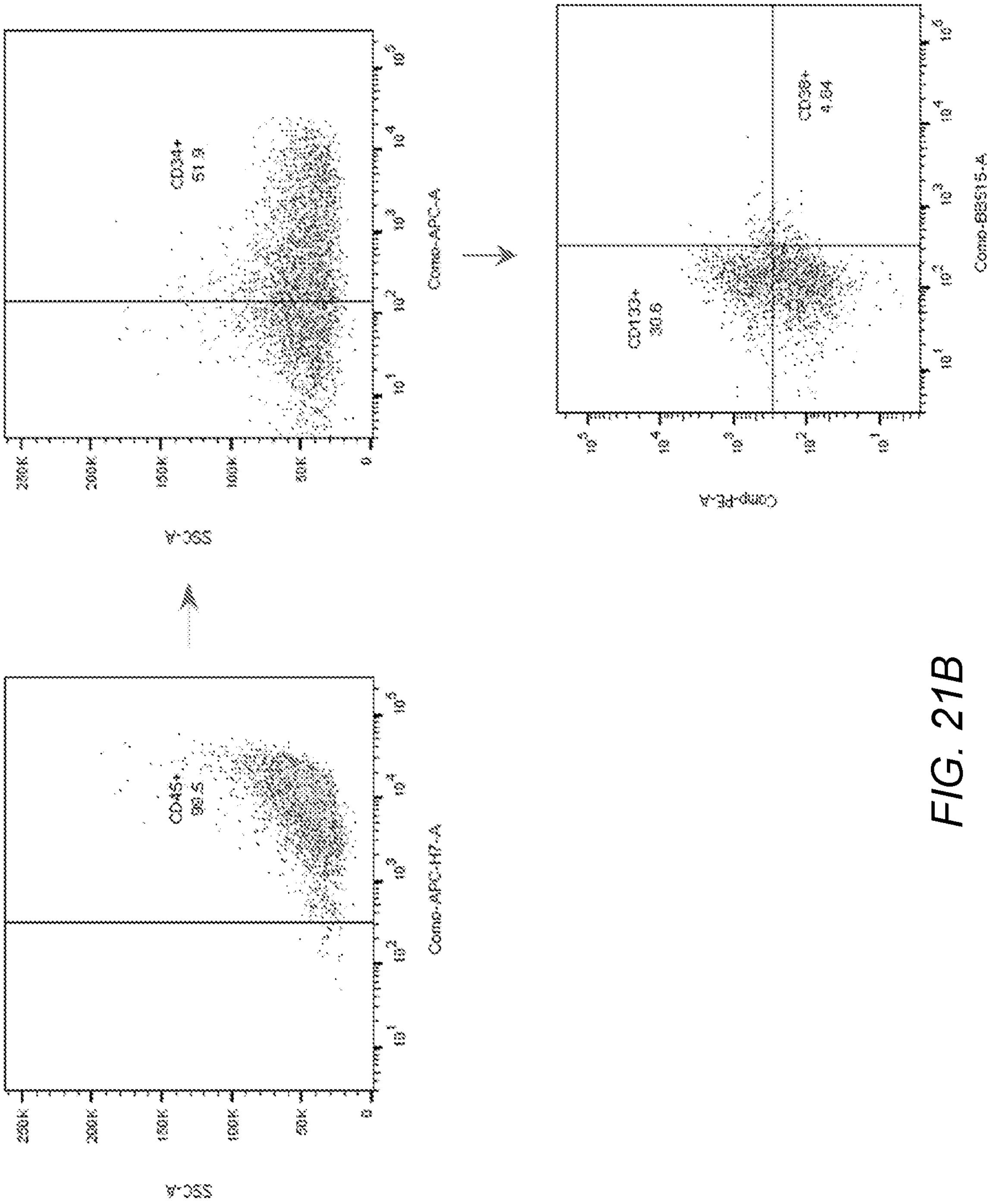
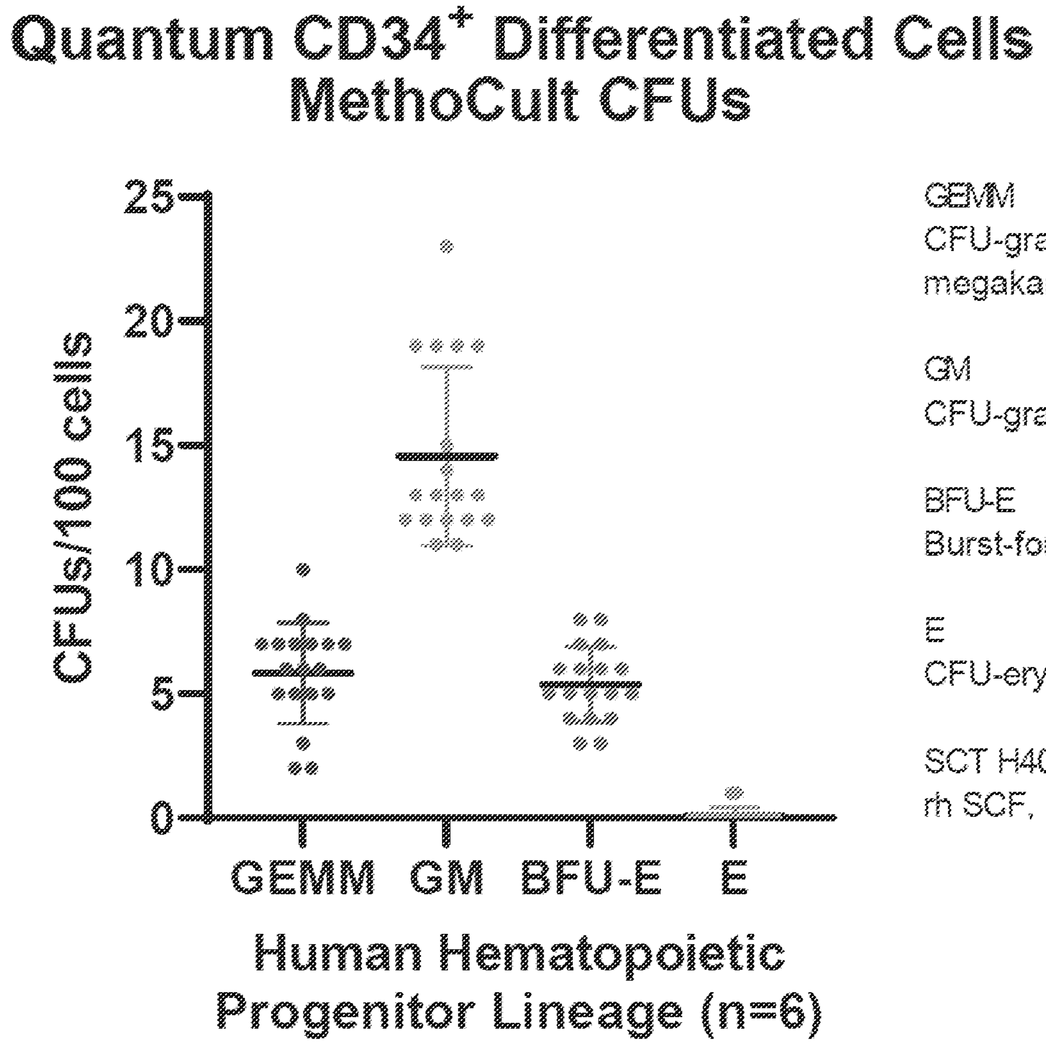

SSC-A
250K
200K
150K
100K
50K
0
10^1
10^2
10^3
10^4
10^5
Comp-APC-H7-A
CD45+
98.5
SSC-A
Comp-APC-A
CD34+
51.9
Comp-PE-A
Comp-BB515-A
CD133+
30.6
CD38+
4.84

FIG. 21B



GEMM
CFU-granulocyte, erythroid, macrophage, megakaryocyte

GM
CFU-granulocyte, macrophage

BFU-E
Burst-forming unit-erythroid

E
CFU-erythroid

SCT H4034 Optimum Media Formulation
rh SCF, rh GM-CSF, rh IL-3, rh G-CSF, rh EPO

FIG. 22

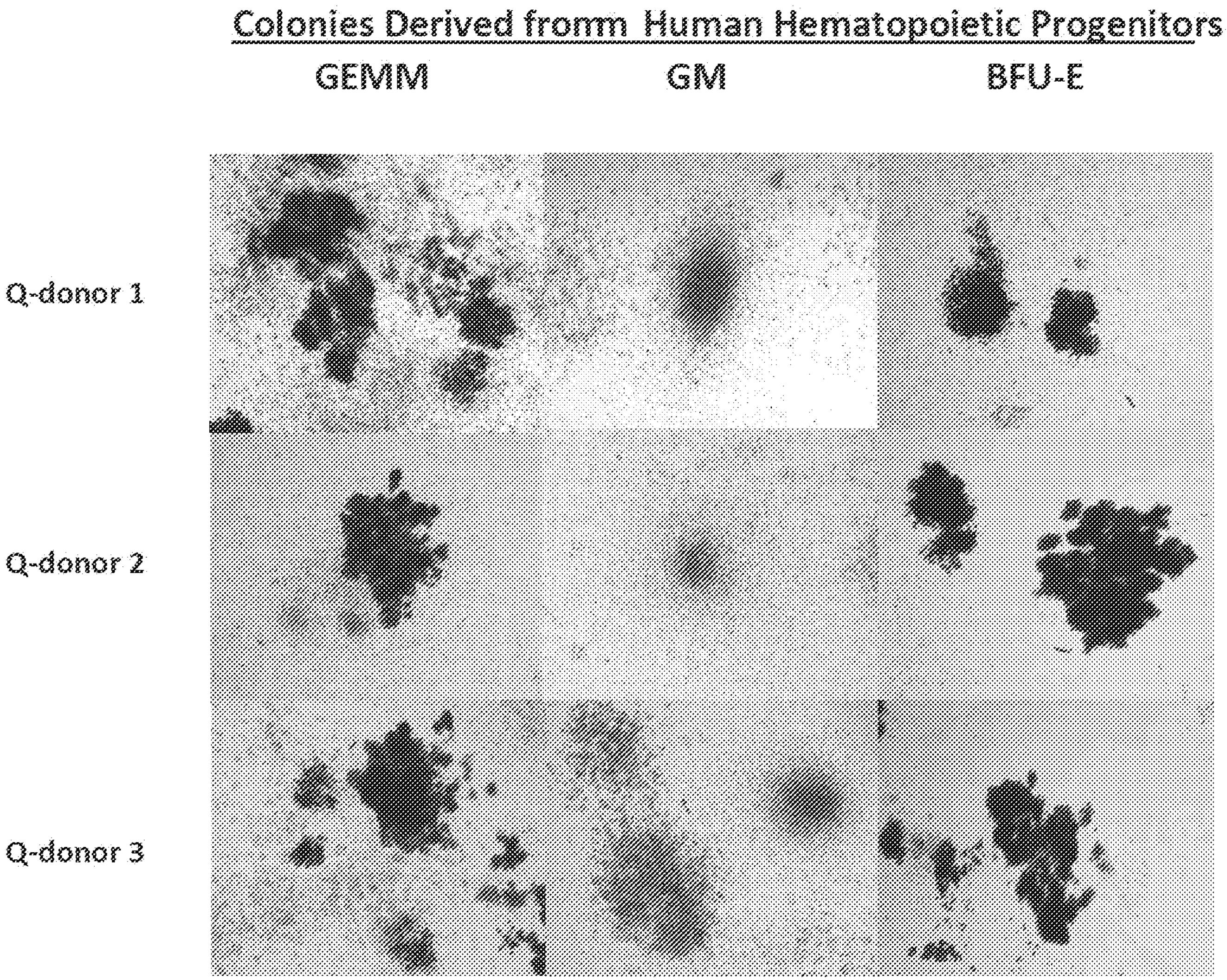

Colonies Derived fromm Human Hematopoietic Progenitors
GEMM
GM
BFU-E
Q-donor 1
Q-donor 2
Q-donor 3

FIG. 23

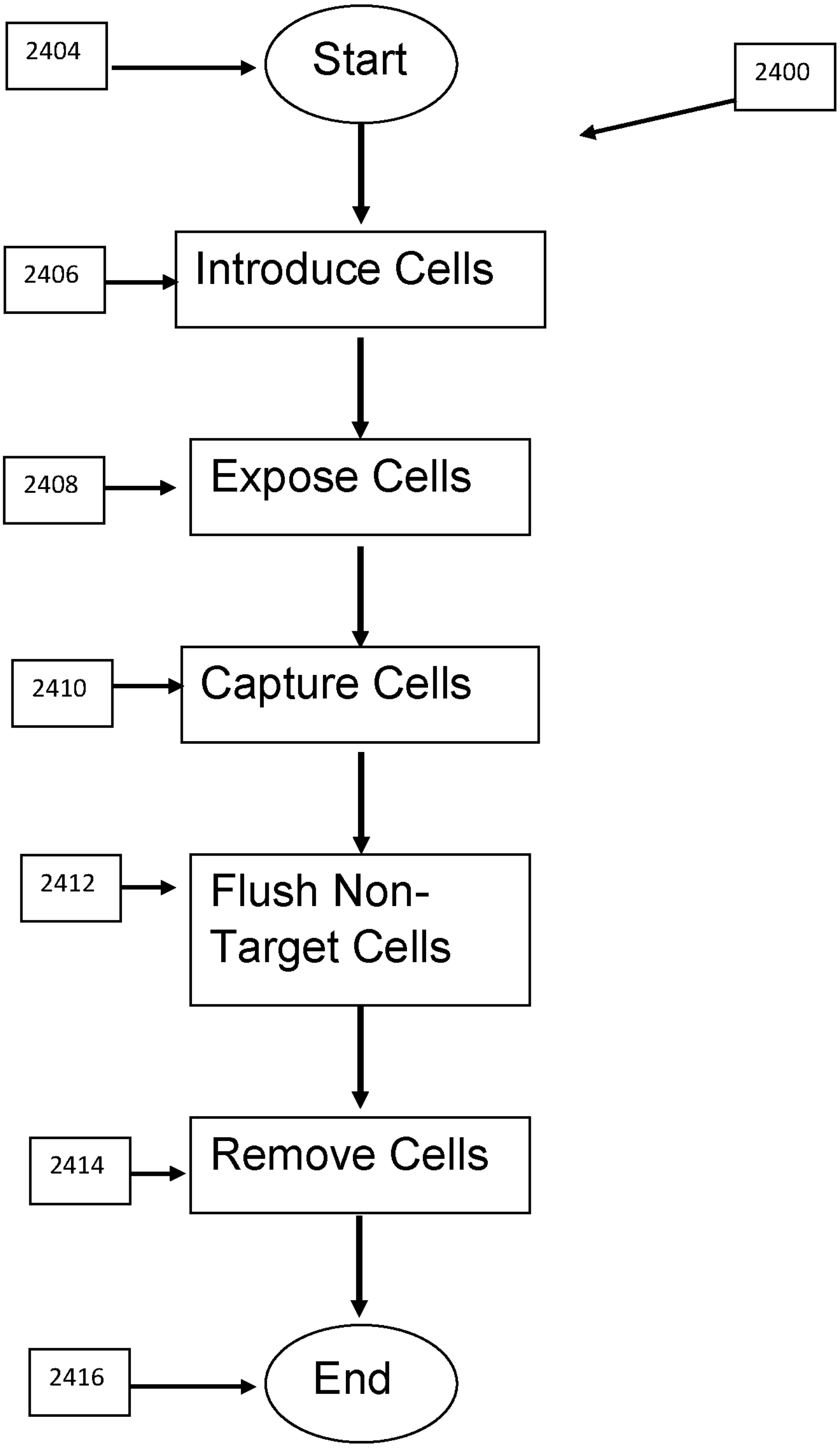

2404
Start
2400
2406
Introduce Cells
2408
Expose Cells
2410
Capture Cells
2412
Flush Non-Target Cells
2414
Remove Cells
2416
End

FIG. 24

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/021595

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N5/00 A61K35/15 C12M1/10 C12M3/00 C12M1/12
C12N5/0789

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N C12M A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CUCHIARA MAUDE L. ET AL: "Covalent immobilization of stem cell factor and stromal derived factor 1[alpha] for in vitro culture of hematopoietic progenitor cells", ACTA BIOMATERIALIA, vol. 9, no. 12, 1 December 2013 (2013-12-01), pages 9258-9269, XP055933720, AMSTERDAM, NL ISSN: 1742-7061, DOI: 10.1016/j.actbio.2013.08.012 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3972068/pdf/nihms516772.pdf> the whole document figures 4,5,7 Materials and methods ----- -/--	1-93

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 June 2022	01/07/2022

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Fax: (+31-70) 340-3016

Authorized officer
Bayer, Martin

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2017/205667 A1 (TERUMO BCT INC [US]) 30 November 2017 (2017-11-30) examples 1-3 claims 1-20 -----	1-93
Y	CHANTAL LECHANTEUR: "Large-Scale Clinical Expansion of Mesenchymal Stem Cells in the GMP-Compliant, Closed Automated Quantum Cell Expansion System: Comparison with Expansion in Traditional T-Flasks", JOURNAL OF STEM CELL RESEARCH & THERAPY, vol. 04, no. 08, 7 August 2014 (2014-08-07), XP055230750, DOI: 10.4172/2157-7633.1000222 the whole document figures 1-3 Materials and methods -----	1-93
Y	FRANK NATHAN D ET AL: "Evaluation of reagents used to coat the hollow-fiber bioreactor membrane of the Quantum Cell Expansion System for the culture of human mesenchymal stem cells", MATERIALS SCIENCE AND ENGINEERING C, ELSEVIER SCIENCE S.A, CH, vol. 96, 26 October 2018 (2018-10-26), pages 77-85, XP085569831, ISSN: 0928-4931, DOI: 10.1016/J.MSEC.2018.10.081 the whole document table 2 Materials and methods -----	1-93
Y	WO 2006/047841 A2 (LEUVEN K U RES & DEV [BE]; ALPHA GEN N V [BE] ET AL.) 11 May 2006 (2006-05-11) example 4 claims 1-29 -----	1-93
X,P	JONES M: "THE MONOCULTURE OF CORD BLOOD-DERIVED CD34+ CELLS BY PERFUSION USING A SEMI-PERMEABLE HOLLOW FIBER MEMBRANE QUANTUM CELL EXPANSION SYSTEM WITH A NOVEL GROWTH FACTOR COCKTAIL", CYTOTHERAPY, vol. 23, no. 5, 25 May 2021 (2021-05-25), page S84, XP055933689, the whole document -----	1-93

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2022/021595

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		WO 2006047841 A2	11-05-2006