

[0019] Currently, the major challenge for clinical cell separation is the robust isolation of rare cell populations with multiple surface markers from a large initial pool of cells. For example, technologies based on centrifugation allow for the isolation of cells from a large initial cell number, and technologies based on MACS can isolate specific populations of cells. However, centrifugation, which pellets cells, often results in physical damage and cell death. In addition, because both centrifugation and MACS techniques involve an open process (i.e., exposed to the environment), microbial contamination of cell separation products remains an issue (Tomlinson MJ et al. J Tissue Eng 4: 2041731412472690).

[0020] Therefore, a need exists for a system and method that is capable of performing a cell-selection process from initial source material to selected cell population while eliminating damage to, and contamination of, the selected cell population. The described invention provides an automated, closed, all-in-one system capable of effectively performing initial cell enrichment, cell labelling, and cell washing, resulting in direct delivery of cells selected based on size. The described invention reduces the risk of human error (i.e., automated), reduces the risk of contamination (i.e., closed system), and prevents damage of the selected cell population (i.e., does not require sedimentation/pelleting of cells).

SUMMARY

[0021] According to the first aspect, the disclosure provides an automated, closed system for selecting a target cell population comprising: an input bag comprising a population of cells suspended in a physiological medium; a chamber embedded in a centrifuge rotor, into which the population of cells is passed; a capture particle injector comprising an agent adapted to identify a subpopulation of the population of cells; to select the subpopulation of the population of cells; and to be released from the subpopulation of the population of cells after the selection; an output bag comprising the released capture particle; the selected cells, or both; and a buffer bag comprising a wash buffer.

[0022] According to one embodiment, the capture particle injector comprises a capture particle adapted to recognize and bind to a cell surface marker on a surface of the subpopulation of the population of cells. According to another embodiment, the capture particle comprises a labeling agent that recognizes and binds to the cell surface marker.

deferoxamine mesylate, iron chelator IV, 21H7; and N,N,N',N'-tetrakis(2-pyridylmethyl)ethane-1,2-diamine (TPEN). According to another embodiment, the calcium chelating agent is ethylenediaminetetraacetic acid (EDTA).

[0031] According to one embodiment, the bead is comprised of a natural polymer. According to another embodiment, the natural polymer is selected from the group consisting of alginate, an alginate derivative, agarose, cross-linked agarose (Sephacrose®), collagen and chitosan. According to another embodiment, the natural polymer is alginate. According to another embodiment, the bead comprises dextran coated with alginate. According to another embodiment, the bead is a microbead.

[0032] According to one embodiment, the antibody is selected from the group consisting of a monoclonal antibody, a polyclonal antibody and a synthetic antibody mimic. According to another embodiment, the monoclonal antibody is selected from the group consisting of a synthetic antibody and an engineered antibody. According to another embodiment, the synthetic antibody is a recombinant antibody. According to another embodiment, the recombinant antibody is selected from the group consisting of a single-chain variable fragment (scFv) antibody, a nucleic acid aptamer and non-immunoglobulin protein scaffold. According to another embodiment, the engineered antibody is selected from the group consisting of a chimeric antibody and a humanized antibody.

[0033] According to another aspect, the disclosure provides a method for isolating a substantially pure population of cells from a heterogeneous cell suspension using the automated, closed system according to the first aspect, comprising: (a) mixing a heterogeneous cell population with capture particles in a chamber embedded in a centrifuge rotor while the rotor is in motion and a counterflow in the chamber produces an opposing force within the chamber, wherein the capture particles comprise a bead conjugated to an agent that recognizes a specific cell surface marker; (b) binding cells to the capture particles in the chamber embedded in the centrifuge rotor while the rotor is in motion and the counterflow produces an opposing force within the chamber, wherein the cells bound to capture particles express the specific cell-surface marker recognized by the agent that recognizes the specific cell surface marker; (c) passing a wash buffer through the chamber embedded in the centrifuge rotor while the rotor is in motion and the counterflow produces an opposing force within the chamber, wherein the wash buffer removes unbound cells and

unbound capture particles from the chamber; (d) collecting the cells bound to the agent that recognizes the specific cell surface marker, wherein the cells bound to the agent that recognizes the specific cell surface marker are enriched relative to the heterogeneous cell suspension; and (e) dissociating the cells in (d) from the agent that recognizes the specific cell surface marker, wherein the method is effective to: reduce the risk of contamination of the collected cells; reduce damage to the collected cells; maintain viability of the collected cells; or a combination thereof.

[0034] According to one embodiment, the bead is comprised of a natural polymer. According to another embodiment, the natural polymer is selected from the group consisting of alginate, an alginate derivative, agarose, cross-linked agarose (Sephacrose®), collagen and chitosan. According to another embodiment, the natural polymer is alginate. According to another embodiment, the bead comprises dextran coated with alginate. According to another embodiment, the bead is a microbead.

[0035] According to one embodiment, the agent that recognizes the specific cell surface marker is an antibody. According to another embodiment, the antibody is selected from the group consisting of a monoclonal antibody, a polyclonal antibody, an engineered antibody, and a synthetic antibody mimic. According to another embodiment, the synthetic antibody mimic is a recombinant antibody. According to another embodiment, the recombinant antibody is selected from the group consisting of a single-chain variable fragment (scFv) antibody, a nucleic acid aptamer and a non-immunoglobulin protein scaffold. According to another embodiment, the engineered antibody is selected from the group consisting of a chimeric antibody and a humanized antibody.

[0036] According to one embodiment, the wash buffer is selected from the group consisting of Tris-buffered saline (TBS), phosphate buffered saline (PBS), Tris-buffered saline-tween-20 (TBST), phosphate-buffered saline-tween-20 (PBST), triethanolamine in PBS and a physiological medium. According to another embodiment, the physiological medium is selected from the group consisting of basal medium eagle (BME), Dulbecco's phosphate buffered saline (DPBS), Dulbecco's modified eagle medium (DMEM), DMEM-F12 media, F-10 nutrient mixture, Glasgow modified minimum essential medium (GMEM), Iscove's modified Delbucco's medium (IMDM), Leibovitz's L-15 medium, McCoy's 5A medium, MCDB 153 medium, media 199, minimal essential medium (MEM), minimal

the group consisting of 100 mM glycine-HCl, pH 2.5-3.0; 100 mM citric acid, pH 3.0; 50-100 mM trimethylamine or triethanolamine, pH 11.5; and 150 mM ammonium hydroxide, pH 10.5. According to another embodiment, the ionic strength solution is selected from the group consisting of 3.5-4.0 M magnesium chloride, pH 7.0 in 10 mM Tris; 5 M lithium chloride in 10 mM phosphate buffer, pH 7.2; 2.5 M sodium iodide, pH 7.5; and 0.2-3.0 M sodium thiocyanate. According to another embodiment, the denaturing solution is selected from the group consisting of 2-6 M guanidine-HCl; 2-8 M urea; 1% deoxycholate; and 1% sodium dodecyl sulfate (SDS). According to another embodiment, the organic solution is selected from the group consisting of 10% dioxane and 50% ethylene glycol, pH 8-11.5.

[0044] According to one embodiment, the method further comprises isolating the labeled targeted subpopulation of cells from the heterogeneous cell population based on size, density, buoyancy or a combination thereof of the labeled targeted subpopulation of cells, wherein the capture particle is effective to alter size, density, buoyancy or a combination thereof of the target cell, and binding of the capture particle comprising the agent that recognizes and binds specifically to the target subpopulation of cells within the heterogeneous cell population is effective to change at least one of size, density and buoyancy of each target cell relative to an unlabeled cell in the heterogeneous cell population.

[0045] According to another aspect, the disclosure provides a method for efficient viral-mediated gene transfer in mammalian cells comprising: (a) Providing a first input bag containing a mammalian cell population and a second input bag containing a transduction buffer comprising a concentrated viral vector that is packaged with genetic material foreign to the mammalian cell population; (b) Adding the first input bag containing the mammalian cell population and the transduction buffer comprising a concentrated viral vector that is packaged with genetic material foreign to the mammalian cell population to a chamber embedded in a centrifuge rotor while the rotor is in motion and a counterflow in the chamber produces an opposing force within the chamber; (c) Incubating the mammalian cell population with the concentrated viral vector packaged with genetic material foreign to the mammalian cell population by circulating the transduction buffer comprising the concentrated viral vector that is packaged with the genetic material of interest around the cells, wherein the incubating is effective to transfer genetic material from the viral vector to a subpopulation of the mammalian cell population to form a transfected subpopulation of mammalian cells; (d) selectively labeling the transfected subpopulation of mammalian cells by

incubating the mammalian cell population with a capture particle comprising an agent that recognizes and binds specifically a cell antigen expressed selectively by the transfected subpopulation within the heterogeneous cell population; binding the capture particle comprising the agent to the targeted population of cells, to form a labeled transfected subpopulation of cells; (e) passing a wash buffer through the chamber embedded in the centrifuge rotor while the rotor is in motion and the counterflow produces an opposing force within the chamber, wherein the wash buffer removes unbound cells and unbound capture particles from the chamber; (f) collecting in an output bag the transfected subpopulation of cells bound to the capture particle comprising the agent that recognizes the specific cell surface marker so that the cells bound to the agent that recognizes the specific cell surface marker are enriched relative to the heterogeneous cell suspension; and (g) dissociating the cells in (f) from the agent that recognizes the specific cell surface marker, wherein the method is effective to: reduce the risk of contamination of the collected cells; reduce damage to the collected cells; maintain viability of the collected cells; or a combination thereof.

[0046] According to one embodiment, binding of the capture particle comprising the agent that recognizes and binds specifically to the transfected subpopulation of cells within the heterogeneous cell population is effective to change at least one of size, density and buoyancy of each transfected cell compared to an unlabeled cell in the heterogeneous cell population.

[0047] According to one embodiment, the method further comprises adding a lysing agent to the chamber embedded in the centrifuge rotor while the rotor is in motion and the counterflow produces an opposing force within the chamber, wherein the lysing agent lyses the bead; and passing a wash buffer through the chamber embedded in the centrifuge rotor while the rotor is in motion and the counterflow produces an opposing force within the chamber, wherein the wash buffer removes the lysing agent and the lysed bead.

[0048] These and other advantages of the disclosure will be apparent to those of ordinary skill in the art by reference to the following detailed description and the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0049] Figure 1 shows a schematic representation of the described invention.