[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 example, 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.

[0021a] In a first aspect, the present disclosure refers to an automated, closed system for selecting a target cell population comprising: (a) an input bag comprising a population of cells suspended in a physiological medium; (b) a chamber embedded in a centrifuge rotor, into which the population of cells is passed; (c) a capture particle injector to inject an agent adapted

a. to identify a subpopulation of the population of cells;
b. to select the subpopulation of the population of cells; and
c. to be released from the subpopulation of the population of cells after the selection;

(d) an output bag comprising the released capture particle; the selected cells, or both; and (e) a buffer bag comprising a wash buffer, the wash buffer removing a cell not bound to the capture particle and a capture particle not bound to a cell from the chamber.

[0022] According to one example, 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 example, the capture particle comprises a labeling agent that recognizes and binds to the cell surface marker.

[0023] According to one example, the automated, closed system further comprises a labelling bag comprising a cell not bound to the capture particle and the capture particle not bound to a cell.

[0024] According to one example, the agent is further conjugated to a bead.

[0025] According to one example, the population of cells is a homogeneous cell population. According to another example, the population of cells is a heterogeneous cell population.

[0026] According to one example, the automated, closed system further comprises a pump.

[0027] According to one example, the chamber is triangular-shaped.

[0028] According to one example, the labeling agent adapted to recognize and bind to the cell-surface marker is an antibody.

[0029] According to one example, 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 example, 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 essential media alpha (MEMA), RPMI 1640 medium, CliniMACS® buffer, Hanks balanced salt saoltion (HBSS), TexMACs™ medium, and Waymouth's MB 752/1 medium.

[0030] According to one example, the automated, closed system further comprises a lysing agent bag comprising a lysing agent that is effective to lyse the bead. According to another example, the lysing agent bag comprises a calcium chelating agent. According to another example, the calcium chelating agent is selected from the group consisting of ethylenediaminetetraacetic acid (EDTA); ethylene glycol tetraacetic acid (EGTA); 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA);

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

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

[0032] According to one example, the antibody is selected from the group consisting of a monoclonal antibody, a polyclonal antibody and a synthetic antibody mimic. According to another example, the monoclonal antibody is selected from the group consisting of a synthetic antibody and an engineered antibody. According to another example, the synthetic antibody is a recombinant antibody. According to another example, 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 example, the engineered antibody is selected from the group consisting of a chimeric antibody and a humanized antibody.

[0033] According to another example, 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 example, 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.

[0033a] In another aspect, the present disclosure relates to 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: (i) reduces the risk of contamination of the collected cells; (ii) reduces damage to the collected cells; (iii) maintains viability of the collected cells; or (iv) a combination thereof.

[0034] According to one example, the bead is comprised of a natural polymer. According to another example, the natural polymer is selected from the group consisting of alginate, an alginate derivative, agarose, cross-linked agarose (Sepharose®), collagen and chitosan. According to another example, the natural

polymer is alginate. According to another example, the bead comprises dextran coated with alginate. According to another example, the bead is a microbead.

[0035] According to one example, the agent that recognizes the specific cell surface marker is an antibody. According to another example, 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 example, the synthetic antibody mimic is a recombinant antibody. According to another example, 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 example, the engineered antibody is selected from the group consisting of a chimeric antibody and a humanized antibody.

[0036] According to one example, 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 example, 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

essential media alpha (MEMA), RPMI 1640 medium, CliniMACS® buffer, Hanks balanced salt saoltion (HBSS), TexMACs™ medium, and Waymouth's MB 752/1 medium.

[0037] According to one example, the method further comprises adding a lysing agent to the chamber embedded in the centrifuge rotor while the rotor is in motion, the counterflow produces an opposing force within the chamber, wherein the lysing agent lyses the bead.

[0038] According to one example, the method further comprises passing a wash buffer through the chamber embedded in the centrifuge rotor while the rotor is in motion, the counterflow producing an opposing force within the chamber, wherein the wash buffer removes the lysing agent and the lysed bead.

[0039] According to one example, the lysing agent is a calcium chelating agent. According to another example, the calcium chelating agent is selected from the group consisting of ethylenediaminetetraacetic acid (EDTA); ethylene glycol tetraacetic acid (EGTA); 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA); deferoxamine mesylate, iron chelator IV, 21H7; and N,N,N',N'-tetrakis(2-pyridylmethy)ethane-1,2-diamine (TPEN). According to another example, the calcium chelating agent is ethylenediaminetetraacetic acid (EDTA).

[0040] According to one example, the collecting is performed by stopping the motion of the centrifuge rotor, increasing rate of the counterflow or a combination thereof.

[0041] According to one example, the contamination is selected from the group consisting of bacterial contamination, viral contamination, fungal contamination and cellular debris.

[0042] According to one example, the damage is selected from the group consisting of cellular swelling, fat accumulation, metabolic failure, structural damage/deterioration and apoptosis.

[0043] According to one example, the dissociating is performed with a dissociation solution. According to another example, the dissociation solution is selected from the group consisting of a pH solution, an ionic strength solution, a denaturing solution and an organic solution. According to another example, the pH solution is selected from

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 example, 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 example, 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 example, the organic solution is selected from the group consisting of 10% dioxane and 50% ethylene glycol, pH 8-11.5.

[0044] According to one example, 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 example, 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.

[0045a] In yet another aspect, the present disclosure refers to 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: (i) reduces the risk of contamination of the collected cells; (ii) reduces damage to the collected cells; (iii) maintains viability of the collected cells; or (iv) a combination thereof.

[0046] According to one example, 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 example, 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.

What is claimed is:

1. An automated, closed system for selecting a target cell population comprising:
    a. an input bag comprising a population of cells suspended in a physiological medium;
    b. a chamber embedded in a centrifuge rotor, into which the population of cells is passed;
    c. a capture particle injector to inject an agent adapted
        a. to identify a subpopulation of the population of cells;
        b. to select the subpopulation of the population of cells; and
        c. to be released from the subpopulation of the population of cells after the selection;
    d. an output bag comprising the released capture particle; the selected cells, or both; and
    e. a buffer bag comprising a wash buffer, the wash buffer removing a cell not bound to the capture particle and a capture particle not bound to a cell from the chamber.

2. The automated, closed system according to claim 1, wherein the capture particle injector injects a capture particle adapted to recognize and bind to a cell surface marker on a surface of the subpopulation of the population of cells.

3. The automated, closed system according to claim 2, wherein the capture particle comprises a labeling agent that recognizes and binds to the cell surface marker.

4. The automated, closed system according to claim 2, further comprising a labelling bag comprising the cell not bound to the capture particle and the capture particle not bound to the cell.

5. The automated, closed system according to claim 2, further comprising a labelling bag comprising a cell bound to a capture particle and a capture particle bound to a cell.

6. The automated, closed system according to claim 1, wherein the agent is further conjugated to a bead.

7. The automated, closed system according to claim 1, wherein the population of cells is a heterogeneous cell population.

8. The automated, closed system according to claim 1, further comprising a pump.

9. The automated, closed system according to claim 1, wherein the chamber is triangular-shaped.

10. The automated, closed system according to claim 3, wherein the labeling agent adapted to recognize and bind to the cell-surface marker is an antibody.

11. The automated, closed system according to claim 1, wherein the wash buffer is selected from the group consisting of Tris-buffered saline (TBS), phosphate buffered saline (PBS), triethanolamine in PBS and a physiological medium.

12. The automated, closed system according to claim 11, wherein 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 essential media alpha (MEMA), RPMI 1640 medium, Hanks balanced salt saoltion (HBSS), and Waymouth's MB 752/1 medium.

13. The automated, closed system according to claim 6, further comprising a lysing agent bag comprising a lysing agent that is effective to lyse the bead.

14. The automated, closed system according to claim 13, wherein the lysing agent bag comprises a calcium chelating agent.

15. The automated, closed system according to claim 14, wherein the calcium chelating agent is selected from the group consisting of ethylenediaminetetraacetic acid (EDTA); ethylene glycol tetraacetic acid (EGTA); 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA); deferoxamine mesylate, iron chelator IV, 21H7; and N,N,N',N'-tetrakis(2-pyridylmethy)ethane-1,2-diamine (TPEN).

16. The automated, closed system according to claim 14, wherein the calcium chelating agent is ethylenediaminetetraacetic acid (EDTA).

17. The automated, closed system according to claim 6, wherein the bead is comprised of a natural polymer.

18. The automated, closed system according to claim 17, wherein the natural polymer is selected from the group consisting of alginate, an alginate derivative, agarose, cross-linked agarose , collagen and chitosan.

19. The automated, closed system according to claim 17, wherein the natural polymer is alginate.

20. The automated, closed system according to claim 6, wherein the bead comprises dextran coated with alginate.

21. The automated, closed system according to claim 6, wherein the bead is a microbead.

22. The automated, closed system according to claim 10, wherein the antibody is selected from the group consisting of a monoclonal antibody, a polyclonal antibody and a synthetic antibody mimic.

23. The automated, closed system according to claim 22, wherein the monoclonal antibody is selected from the group consisting of a synthetic antibody and an engineered antibody.

24. The automated, closed system according to claim 23, wherein the synthetic antibody is a recombinant antibody.

25. The automated, closed system according to claim 24, wherein 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.

26. The automated, closed system according to claim 23, wherein the engineered antibody is selected from the group consisting of a chimeric antibody and a humanized antibody.

27. A method for isolating a substantially pure population of cells from a heterogeneous cell suspension using the automated, closed system according to claim 1, 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:

(i) reduces the risk of contamination of the collected cells;

(ii) reduces damage to the collected cells;

(iii) maintains viability of the collected cells; or

(iv) a combination thereof.

28. The method according to claim 27, wherein the bead is comprised of a natural polymer.

29. The method according to claim 28, wherein the natural polymer is selected from the group consisting of alginate, an alginate derivative, agarose, cross-linked agarose , collagen and chitosan.

30. The method according to claim 28, wherein the natural polymer is alginate.

31. The method according to claim 27, wherein the bead comprises dextran coated with alginate.

32. The method according to claim 27, wherein the bead is a microbead.

33. The method according to claim 27, wherein the agent that recognizes the specific cell surface marker is an antibody.

34. The method according to claim 33, wherein the antibody is selected from the group consisting of a monoclonal antibody, a polyclonal antibody, an engineered antibody, and a synthetic antibody mimic.

35. The method according to claim 34, wherein the synthetic antibody mimic is a recombinant antibody.

36. The method according to claim 35, wherein 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.

37. The method according to claim 34, wherein the engineered antibody is selected from the group consisting of a chimeric antibody and a humanized antibody.

38. The method according to claim 27, wherein the wash buffer is selected from the group consisting of Tris-buffered saline (TBS), phosphate buffered saline (PBS), triethanolamine in PBS and a physiological medium.

39. The method according to claim 38, wherein 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 essential media alpha (MEMA), RPMI 1640 medium, Hanks balanced salt saoltion (HBSS), and Waymouth's MB 752/1 medium.

40. The method according to claim 27, further comprising

adding a lysing agent to the chamber embedded in the centrifuge rotor while the rotor is in motion, the counterflow produces an opposing force within the chamber, wherein the lysing agent lyses the bead.

41. The method according to claim 40, further comprising

passing a wash buffer through the chamber embedded in the centrifuge rotor while the rotor is in motion, the counterflow producing an opposing force within the chamber, wherein the wash buffer removes the lysing agent and the lysed bead.

42. The method according to claim 40, wherein the lysing agent is a calcium chelating agent.

43. The method according to claim 42, wherein the calcium chelating agent is selected from the group consisting of ethylenediaminetetraacetic acid (EDTA); ethylene glycol tetraacetic acid (EGTA); 1,2-bis(o-aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid (BAPTA); deferoxamine mesylate, iron chelator IV, 21H7; and N,N,N’,N’-tetrakis(2-pyridylmethy)ethane-1,2-diamine (TPEN).

44. The method according to claim 42, wherein the calcium chelating agent is ethylenediaminetetraacetic acid (EDTA).

45. The method according to claim 27, wherein the collecting in (d) is performed by stopping the motion of the centrifuge rotor, increasing rate of the counterflow or a combination thereof.

46. The method according to claim 27, wherein the contamination is selected from the group consisting of bacterial contamination, viral contamination, fungal contamination and cellular debris.

47. The method according to claim 27, wherein the damage is selected from the group consisting of cellular swelling, fat accumulation, metabolic failure, structural damage/deterioration and apoptosis.

48. The method according to claim 27, wherein the dissociating in (e) is performed with a dissociation solution.

49. The method according to claim 48, wherein the dissociation solution is selected from the group consisting of a pH solution, an ionic strength solution, a denaturing solution and an organic solution.

50. The method according to claim 49, wherein the pH solution is selected from 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.

51. The method according to claim 49, wherein 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.

52. The method according to claim 49, wherein 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).

53. The method according to claim 49, wherein the organic solution is selected from the group consisting of 10% dioxane and 50% ethylene glycol, pH 8-11.5.

54. The method according to claim 27, further comprising 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

(a) the capture particle alters size, density, buoyancy or a combination thereof of the target cell, and

(b) binding of the capture particle comprising the agent that recognizes and binds specifically to the target subpopulation of cells within the heterogeneous cell population changes at least one of size, density and buoyancy of each target cell relative to an unlabeled cell in the heterogeneous cell population.

55. 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

    (i) 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;

    (ii) 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:

(i) reduces the risk of contamination of the collected cells;
(ii) reduces damage to the collected cells;
(iii) maintains viability of the collected cells; or
(iv) a combination thereof.

56. The method according to claim 55, wherein binding of the capture particle comprising the agent that recognizes and binds specifically to the transfected subpopulation of cells within the heterogeneous cell population changes at least one of size, density and buoyancy of each transfected cell compared to an unlabeled cell in the heterogeneous cell population.

57. The method according to claim 55, further comprising

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.