

WHAT IS CLAIMED IS:

1. A bioprocessing method for cell therapy, the method comprising the steps of:
genetically modifying a population of cells in a bioreactor vessel to produce a population of genetically modified cells; and
expanding the population of genetically modified cells within the bioreactor vessel to generate a number of genetically modified cells sufficient for one or more doses for use in a cell therapy treatment without removing the population of genetically modified cells from the bioreactor vessel.
2. The bioprocessing method according to claim 1, further comprising the steps of:
activating a population of cells in the bioreactor vessel prior to genetically modifying the cells;
wherein the steps of activating, genetically modifying, and expanding are performed without removing cells from the bioreactor vessel.
3. The bioprocessing method according to claim 1, further comprising the step of:
washing the population of cells prior to the step of genetically modifying;
concentrating the cells within the bioreactor by fluid removal after the activating step and prior to genetically modifying;
wherein the steps of washing and concentrating are carried out without removing cells from the bioreactor vessel.
4. The bioprocessing method according to claim 1, further comprising, during the expanding step, perfusing the population of cells with media via filterless perfusion
5. The bioprocessing method according to claim 1, wherein genetically modifying a population of cells comprises genetically modifying cells with a genetic modification agent selected from viruses, viral vectors, and non-viral vectors.

6. The bioprocessing method according to claim 5, further comprising the step of:
washing the genetically modified cells in the bioreactor vessel to remove the genetic modification agent and/or unwanted soluble materials via filterless perfusion.
7. The bioprocessing method according to claim 5, wherein the viral vector is a lentivirus or a retrovirus or an adeno-associated virus.
8. The bioprocessing method according to claim 1, wherein:
the population of cells is a first population of cells; and
further comprising the steps of:
genetically modifying a second population of cells in a second bioreactor vessel to produce a second population of genetically modified cells; and
expanding the second population of genetically modified cells within the second bioreactor without removing the second population of genetically modified cells from the second bioreactor vessel.
9. The bioprocessing method according to claim 8, wherein:
the bioreactor vessel is at least an interconnected first and second bioreactor vessel interconnected through at least one fluid flow line; and
further comprising the steps of:
activating the cells in a first bioreactor vessel interconnected with the second bioreactor vessel; and
transferring the cells to the second vessel through the at least one fluid flow line.
10. The bioprocessing method according to claim 8, wherein the first population of cells and the second population of cells are from the same donor source.
11. The bioprocessing method according to claim 8, wherein the first population of cells and the second population of cells are from different donor sources.

12. The bioprocessing method according to claim 8, wherein the first population of cells is enriched for a first subpopulation of cells and the second population of cells is enriched for a second subpopulation of cells, wherein the first subpopulation of cells is different from the second subpopulation of cells.
13. The bioprocessing method according to claim 1, wherein the cells are selected from immune cells, T cells, B cells, dendritic cells, CAR-T cells, TCR-T cells, NK cells, and combinations thereof.
14. A bioprocessing method comprising the steps of:
 - coating a bioreactor vessel with a reagent for enhancing efficiency of genetic modification of a population of cells;
 - genetically modifying cells of a population of cells to produce a population of genetically modified cells; and
 - expanding the population of genetically modified cells in the bioreactor vessel without removing the genetically modified cells from the bioreactor vessel.
15. The bioprocessing method according to claim 14, wherein the bioreactor vessel is coated at a temperature of about 37°C.
16. The bioprocessing method according to claim 14, wherein the reagent is selected from Retronectin, fibronectin, a fibronectin fragment, a reagent with a heparin binding domain and a cell binding domain, and combinations thereof.
17. The bioprocessing method according to claim 14, wherein:
 - the step of expanding the population of genetically modified cells includes culturing over Retronectin or fibronectin or a fragment of fibronectin or a reagent with a heparin binding domain and a cell binding domain for a predetermined duration.
18. A bioprocessing method comprising

activating cells of a population of cells in a bioreactor vessel using magnetic or non-magnetic beads to produce a population of activated cells;
genetically modifying the activated cells in the bioreactor vessel to produce a population of genetically modified cells;
washing the genetically modified cells in the bioreactor vessel to remove unwanted materials; and
expanding the population of genetically modified cells in the bioreactor vessel to produce an expanded population of transduced cells;
wherein activating, genetically modifying, washing, and expanding are carried out in the bioreactor vessel without removing the cells from the bioreactor vessel.

19. The bioprocessing method according to claim 18, wherein:
the beads are retained in the bioreactor vessel during the step of washing the genetically modified cells.
20. The bioprocessing method according to claim 18, wherein:
the step of washing is carried out without immobilizing magnetic isolation beads using a magnet.
21. The bioprocessing method according to claim 18, additionally comprising washing the genetically modified cells via filterless perfusion.
22. The bioprocessing method according to claim 18, further comprising the steps of:
harvesting the expanded population of genetically modified cells from the bioreactor vessel; and
debeading the expanded population of genetically modified cells.
23. The bioprocessing method according to claim 22, wherein:
the steps of harvesting the expanded population of genetically modified cells and
debeading the expanded population of genetically modified cells are carried out simultaneously.

24. The bioprocessing method according to claim 22, wherein:
the steps of harvesting and debeaded the expanded population of genetically modified cells are carried out using perfusion: and
during perfusion the magnetic beads remain in the bioreactor vessel.
25. The bioprocessing method according to claim 18, wherein:
the cells are one or more of immune cells, T-cells, T cell subsets, B cells, NK cells and dendritic cells.
26. The bioprocessing method according to any of claims 1, 14 or 18, wherein the bioreactor vessel comprises a first bioreactor vessel and a second bioreactor vessel disposed within a processing chamber of a bioprocessing apparatus.
27. The bioprocessing method according to claim 26, wherein the steps of activating and genetically modifying are performed in the first bioreactor vessel, and the step of expanding is performed in the second bioreactor vessel.
28. A bioprocessing method optionally as claimed in any one of the preceding claims, comprising:
introducing a suspension comprising cells suspended in a cell culture medium through at least a feed port or a drain port into a cavity of a cell culture vessel, the suspension being in an amount sufficient to cover a gas permeable, liquid impermeable membrane positioned at a bottom of the cell culture vessel, the feed port configured to permit additional cell culture medium into the cavity, and the drain port configured to permit removal of the cells, cell culture medium, and used cell culture medium from the cavity;
allowing the cells to settle on the gas permeable, liquid impermeable membrane by gravity;
removing the used cell culture medium through the drain port and introducing the additional cell culture medium through the feed port such that a constant volume is maintained in the cell culture vessel until the cells expand to a desired density, wherein the removing and

introducing are preformed subsequent to allowing the cells to settle on the gas permeable, liquid impermeable membrane;
resuspending the cells in the cell culture medium in the cell culture vessel, wherein the resuspending is performed after the desired cell density is attained; and
removing the resuspended cells and the cell culture medium through the drain port.

29. The method of claim 28, wherein removing the used cell culture medium comprises removing impurities, cell culture byproducts, or a combination thereof from within the cell culture vessel.

30. The method of claim 28 or 29, wherein the allowing the cells to settle to the gas permeable, liquid impermeable membrane, the removing the used cell culture medium, and the introducing the additional cell culture medium comprise positioning the cell culture vessel such that the gas permeable, liquid impermeable membrane is level to allow even distribution of cells.

31. The method of claim 28, 29 or 30, wherein the resuspending of the cells in the cell culture medium comprises oscillating the cell culture vessel through a defined angle for a period of time.

32. The method of any one of the preceding claims 28 to 31, wherein the removing of the resuspended cells and the cell culture medium through the drain port comprises tilting the cell culture vessel to a low angle to minimize a hold-up volume during removal while maintaining a low overall profile of the cell culture vessel.

33. A bioprocessing method, comprising:
introducing a suspension comprising cells suspended in a cell culture medium through at least a feed port or a drain port into a cavity of a cell culture vessel, the suspension being in an amount sufficient to cover a gas permeable, liquid impermeable membrane positioned at a bottom of the cell culture vessel, the feed port being disposed through a surface of the cell culture vessel and configured to permit additional cell culture medium into the cavity, and the

drain port being disposed through a surface of the cell culture vessel and configured to permit removal of the cells, cell culture medium, and used cell culture medium from the cavity;

allowing the cells to settle on the gas permeable, liquid impermeable membrane by gravity;

perfusing the cells with the cell culture medium, wherein the perfusing comprises introducing the additional cell culture medium through the feed port while simultaneously or nearly simultaneously removing the used cell culture medium from the cell culture vessel via the drain port to maintaining a constant volume within the cell culture vessel until the cells expand to a desired cell density, wherein the perfusing is performed subsequent to allowing the cells to settle on the gas permeable, liquid impermeable membrane;

sampling the cells via the drain port to determine whether the desired cell density is reached;

repeating, upon determining that the desired cell density has not been reached, the perfusing of the cells until the desired cell density is reached;

resuspending the cells in the cell culture medium in the cell culture vessel, wherein the resuspending is performed after the desired cell density is reached; and
removing the resuspended cells and the cell culture medium through the drain port.

34. A cell culture vessel for expansion of a cell density, comprising:

a bottom plate comprising a grid surface configured to provide support and gas exchange;

a vessel body coupled to the bottom plate, wherein the vessel body comprises a rigid concave structure;

a cavity formed by the vessel body and the bottom plate;

a gas-permeable, liquid impermeable membrane disposed above the bottom plate within the cavity;

a feeding port disposed through a surface of the vessel body, wherein the feeding port is configured to permit addition of cell culture medium into the cavity; and

a drain port disposed through a wall of the vessel body, wherein the drain port is configured to permit removal of used cell culture medium from the cavity;

wherein the cell culture vessel is configured to hold a suspension comprising cells suspended in the cell culture medium, and wherein the cell culture medium within the cell

culture vessel comprises a ratio of cell culture medium volume per surface area of the gas permeable, liquid impermeable membrane that is below a predetermined threshold value.