Summary

[0003a] In one aspect, the present invention provides a transduction method, comprising incubating, in an internal cavity of a centrifugal chamber, an input composition comprising cells and viral particles containing a recombinant viral vector, wherein

said centrifugal chamber comprises:

an end wall, a substantially rigid side wall extending from said end wall, and one or more opening, wherein at least a portion of said side wall surrounds said internal cavity and at least one of the one or more opening is capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity;

a movable member capable of moving within the chamber to vary the internal volume of the cavity, whereby the internal cavity is a cavity of variable volume defined by said end wall, said substantially rigid side wall, and said movable member;

the centrifugal chamber is rotatable around an axis of rotation and is rotating around said axis of rotation during at least a portion of the incubation; and

the method generates an output composition comprising a plurality of the cells transduced with the viral vector.

[0004] Provided are methods for cell processing, such as for transfer of viral vectors and/or immunoaffinity-based selection of cells. In some examples, the cells are for use in cell therapy, such primary cells prepared for autologous or allogeneic transfer, e.g., in adoptive cell therapy. The methods may include additional cell processing steps, such as cell washing, isolation, separation.

[0005] In some examples, the methods are carried out by incubating, in a vessel, such as an internal cavity of a centrifugal chamber, a composition (deemed an input composition), which contains cells and viral vector particles, the viral particles containing a recombinant viral vector, thereby generating an output composition that contains a plurality of the cells transduced with the viral vector. The centrifugal chamber typically is rotatable around an axis of rotation. The axis of rotation in some examples is vertical. The chamber typically includes an end wall, a side wall extending from the end wall, such as a substantially rigid side wall, and at least one opening, such as an inlet/outlet or an inlet and an outlet. At least a portion of the side wall generally surrounds the internal cavity. The at least one opening (e.g., the inlet/outlet or the inlet and the outlet) is capable of permitting intake of liquid into the internal cavity and expression of liquid from the cavity. The at least one opening in some examples is coaxial with the chamber and in some examples is

in an end wall of the chamber. The side wall may be a curvilinear, e.g., cylindrical or generally cylindrical.

[0006] In some examples, the methods include incubating, in an internal cavity of a centrifugal chamber, an input composition containing cells and viral particles containing a recombinant viral vector, wherein said centrifugal chamber is rotatable around an axis of rotation and includes an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, at least a portion of said side wall surrounding said internal cavity and said at least one opening being capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity, wherein the centrifugal chamber is rotating around said axis of rotation during at least a portion of the incubation and the method generates an output composition containing a plurality of the cells transduced with the viral vector.

[0007] In some examples, the centrifugal chamber further includes a movable member, such as a piston. In such examples, the internal cavity is generally one of variable volume, e.g., a cavity of variable volume defined by the end wall, the side wall, and the movable member, e.g., the piston, such that the movable member is capable of moving within the chamber (such as axially within the chamber) to vary the internal volume of the cavity. In some examples,

liquid is moved in and out of the chamber alternatively by way of a pump, syringe, and/or motor, or other device for intake and expressing liquid or gas, which for example pulls liquid from the cavity and/or pushes liquid in, while the volume of the cavity itself remains constant.

[0008] In some examples, the methods include incubating, in an internal cavity of a centrifugal chamber, an input composition containing cells and a viral particle containing a recombinant viral vector, said centrifugal chamber being rotatable around an axis of rotation and comprising an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, wherein at least a portion of said side wall surrounds said internal cavity and said at least one opening is capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity, wherein the centrifugal chamber is rotating around the axis of rotation during at least a portion of the incubation, the total liquid volume of said input composition present in said cavity during rotation of said centrifugal chamber is no more than about 5 mL per square inch of the internal surface area of the cavity and the method generates an output composition comprising a plurality of the cells transduced with the viral vector.

[0009] The chamber may comprise two end walls. In some such examples, one end wall together with other features defines the internal cavity, while the other is outside of the cavity. In some examples, the cavity is bound by both end walls.

[0010] The at least one opening may comprise: an inlet and an outlet, respectively capable of permitting said intake and expression, or a single inlet/outlet, capable of permitting said intake and said expression.

[0011] Typically, the incubation is carried out at least in part under rotation of the chamber, such as under centrifugal force or acceleration. Thus, the methods in some examplesfurther include effecting rotation of the centrifugal chamber, such as around its axis of rotation, during at least a portion of the incubation.

[0012] In some of any such examples, said rotating includes rotation at a relative centrifugal force (RCF) at an internal surface of the side wall of the cavity and/or at a surface layer of the cells of greater than at or about 200 g, greater than at or about 300 g, or greater than at or about 500 g. In some of any such examples, said rotating includes rotation at a relative centrifugal force at an internal surface of the side wall of the cavity and/or at a surface layer of the cells that is: at or about 1000 g, 1500 g, 2000 g, 2100 g, 2200 g, 2500 g or 3000 g; or at least at or about 1000 g, 1500 g, 2000 g, 2100 g, 2200 g, 2500 g, or 3000 g. In some of any such examples, said rotating includes rotation at a relative centrifugal force at an internal surface of the side wall of the cavity and/or at a surface layer of the cells that is: between or between

about 1000 and 3600, 1000 and 3200, 1000 and 2800, 1000 and 2000, 1000 and 1600, 1600 and 3600, 1600 and 3200, 1600 and 2800, 1600 and 2000, 2000 and 3600, 2000 and 3200, 2000 and 2800, 2800 and 3600, 2800 and 3200, 3200 and 3600, each inclusive; or at least at or about 2000 g, 2100, 2200 g, 2400 g, 2600g, 2800 g, 3000 g, 3200 g or 3600 g; or at or about 2000 g, 2100g, 2200 g, 2400 g, 2600g, 2800 g, 3000 g, 3200 g or 3600 g.

[0013] In some of any such examples, the at least a portion of the incubation during which the chamber is rotating is for a time that is: greater than or about 5 minutes, such as greater than or about 10 minutes, greater than or about 15 minutes, greater than or about 20 minutes, greater than or about 30 minutes, greater than or about 45 minutes, greater than or about 60 minutes, greater than or about 90 minutes or greater than or about 120 minutes; or between or between about 5 minutes and 60 minutes, 10 minutes and 60 minutes, 15 minutes and 60 minutes, 15 minutes and 45 minutes, 30 minutes and 60 minutes or 45 minutes and 60 minutes, each inclusive.

[0014] In some examples, the input composition (or the number of cells) in the cavity during the incubation, e.g., at any one time or during the entire incubation, and/or processed by the methods, includes at or about or at least about 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8 or 5×10^8 of the cells.

[0015] In some of any such examples, said input composition in the cavity contains at least at or about 1×10^7 of said cells, at least at or about 2×10^7 of said cells, 3×10^7 of said cells, at least at or about 4×10^7 of said cells, at least at or about 5×10^7 of said cells, at least at or about 6×10^7 of said cells, at least at or about 7×10^7 of said cells, at least at or about 8×10^7 of said cells, at least at or about 9×10^7 of said cells, at least at or about 1×10^8 of said cells, at least at or about 2×10^8 of said cells, at least at or about 3×10^8 of said cells or at least at or about 4×10^8 of said cells.

[0016] In some examples, the internal surface area of the cavity is at least at or about 1×10^9 μm^2 or 1×10^{10} μm^2, and/or the length of the side wall in the direction extending from the end wall is at least about 5 cm and/or at least about 8 cm; and/or the internal cavity has a radius of at least about 2 cm at at least one cross-section.

[0017] In some examples, the input composition includes at least or about 1 infectious unit (IU) per one of the cells, at least or about 2 IU per one of the cells, at least or about 3 IU per one of the cells, at least or about 4 IU per one of the cells, at least or about 5 IU per one of the cells, at least or about 10 IU per one of the cells, at least or about 20 IU per one of the cells, at least or about 30 IU per one of the cells, at least or about 40 IU per one of the cells, at least or

about 50 IU per one of the cells, or at least or about 60 IU per one of the cells. In some examples, the input composition includes at or about 1 infectious unit (IU) per one of the cells, at or about 2 IU per one of the cells, at or about 3 IU per one of the cells, at or about 4 IU per one of the cells, at or about 5 IU per one of the cells, at or about 10 IU per one of the cells, at or about 20 IU per one of the cells, at or about 30 IU per one of the cells, at or about 40 IU per one of the cells, at or about 50 IU per one of the cells, or at or about 60 IU per one of the cells.

[0018] In some examples, the average liquid volume or maximum liquid volume of the input composition, composition with viral vector particles and cells, and/or any liquid composition present in the cavity during the incubation is no more than about 10, 5, or 2.5 milliliters (mL) per square inch of the internal surface area of the cavity during the incubation. In some examples, the maximum total volume of such liquid composition present in the cavity at any one time during the incubation is no more than 2 times, no more than 10 times, no more than 100 times, no more than 500 times or no more than 1000 times the total volume of the cells. In some examples, the total volume of cells is the total volume of a pellet of the cells. In some examples, the total volume of cells is the volume of a monolayer of the cells, such as a monolayer of cells present on the internal surface in the cavity during rotation of the centrifugal chamber.

[0019] In some examples, the liquid volume of the input composition occupies all or substantially all of the volume of the internal cavity during at least a portion of the incubation. In other examples, during at least a portion of the incubation, the liquid volume of the input composition occupies only a portion of the volume of the internal cavity, the volume of the cavity during this at least a portion further comprising a gas, which is taken into the cavity, e.g., via said at least one opening or another opening, prior to or during the incubation.

[0020] In some of any such examples, the liquid volume of said input composition present in said cavity during said rotation is between or between about 0.5 mL per square inch of the internal surface area of the cavity (mL/sq.in) and 5 mL/sq.in, 0.5 mL/sq.in. and 2.5 mL/sq.in., 0.5 mL/sq.in. and 1 mL/sq.in., 1 mL/sq.in. and 5 mL/sq.in., 1 mL/sq.in. and 2.5 mL/sq.in. or 2.5 mL/sq.in. and 5 mL/sq.in.

[0021] In some of any such examples, the maximum total liquid volume of said input composition present in said cavity at any one time during said incubation is no more than 2 times, no more than 10 times, or no more than 100 times, the total volume of said cells in said cavity or the average volume of the input composition over the course of the incubation is no more than 2, 10, or 100 times the total volume of cells in the cavity.

[0022] In some of any such examples, the maximum volume of said input composition present in said cavity at any one time during said incubation or the average volume over the course of the incubation is no more than at or about 2 times, 10 times, 25 times, 50 times, 100 times, 500 times, or 1000 times the volume of a monolayer of said cells formed on the inner surface of said cavity during rotation of said chamber at a force of at or about 2000 g at an internal surface of the side wall.

[0023] In some of any such examples, the liquid volume of the input composition is no more than 20 mL, no more than 40 mL, no more than 50 mL, no more than 70 mL, no more than 100 mL, no more than 120 mL, no more than 150 mL or no more than 200 mL.

[0024] In some of any such examples, during at least a portion of the incubation in the chamber or during the rotation of the chamber, the liquid volume of the input composition occupies only a portion of the volume of the internal cavity of the chamber, the volume of the cavity during said at least a portion or during said rotation further comprising a gas, said gas taken into said cavity via said at least one opening, prior to or during said incubation.

[0025] In some examples, the centrifugal chamber includes a movable member, whereby intake of gas into the centrifugal chamber effects movement of the movable member to increase the volume of the internal cavity of the chamber, thereby decreasing the total liquid volume of said input composition present in said cavity during rotation of said centrifugal chamber per square inch of the internal surface area of the cavity compared to the absence of gas in the chamber.

[0026] In some examples, the number of cells in the cavity during the incubation is at or about the number of the cells sufficient to form a monolayer on the internal surface of the cavity during rotation of the centrifugal chamber at a force of at or about 2000 g and/or is no more than 1.5 times or 2 times such a number of the cells.

[0027] In some of any such examples, the number of said cells in said input composition is at or about the number of said cells sufficient to form a monolayer on the surface of said cavity during rotation of said centrifugal chamber at a force of at or about 2000 g at an internal surface of the side wall; and/or the number of said cells in said input composition is no more than 1.5 times or 2 times the number of said cells sufficient to form a monolayer on the surface of said cavity during rotation of said centrifugal chamber at a force of at or about 2000 g at an internal surface of the side wall.

[0028] In some examples, the centrifugation is for a duration of between 120 and 7200 seconds, such as between 120 and 3600 seconds, including values inclusive or within the range, such as whole-minute values inclusive or within the range.

[0029] In some examples, the methods include a) providing to an internal cavity of a centrifugal chamber that has an internal surface area of at least at or about 1×10^9 μm^2 or at least at or about 1×10^{10} μm^2: i) an input composition including cells and viral particles containing a recombinant viral vector, wherein: the number of cells in the input composition is at least 1×10^7 cells, and the viral particles are present in the input composition at at least at or about 1 infectious unit (IU) per one of said cells, and the input composition contains a liquid volume that is less than the maximum volume of the internal cavity of the centrifugal chamber; and ii) gas, at a volume that is up to the remainder of the maximum volume of the internal cavity of the centrifugal chamber; and b) incubating the input composition, wherein at least a portion of the incubation is carried out in said internal cavity of said centrifugal chamber while effecting rotation of said centrifugal chamber; and wherein the method generates an output composition containing a plurality of the cells transduced with the viral vector.

[0030] In some examples, the number of cells is at least or about 50×10^6 cells; 100×10^6 cells; or 200×10^6 cells; and/or the viral particles are present at at least 1.6 IU/cell, 1.8 IU/cell, 2.0 IU/cell, 2.4 IU/cell, 2.8 IU/cell, 3.2 IU/cell or 3.6 IU/cell, 4.0 IU/cell, 5.0 IU/cell, 6.0 IU/cell, 7.0 IU/cell, 8.0 IU/cell, 9.0 IU/cell or 10.0 IU/cell.

[0031] In some of any such examples, the liquid volume of the input composition is less than or equal to 200 mL, less than or equal to 100 mL or less than or equal to 50 mL or less than or equal to 20 mL. In some of any such examples, the volume of gas is up to 200 mL, up to 180 mL, up to 140 mL or up to 100 mL.

[0032] In some of any such examples, said rotation is at a relative centrifugal force at an internal surface of the side wall of the cavity or at a surface layer of the cells of at least at or about 1000 g, 1500 g, 2000 g, 2400 g, 2600g, 2800 g, 3000 g, 3200 g or 3600 g.

[0033] In some examples, the methods are for large-scale processing.

[0034] In some examples, the composition in the cavity (e.g., input composition) includes at least 50 mL, at least 100 mL, or at least 200 mL, liquid volume, and/or at least or about 1 million cells per cm^2 of the internal surface area of the cavity during at least a portion of said incubation.

[0035] In some examples, the maximum liquid volume of the input composition present in the cavity at any one time during said incubation is no more than at or about 2 times, 10 times,

25 times, 50 times, 100 times, 500 times, or 1000 times the volume of a monolayer of said cells formed on the inner surface of said cavity during rotation of said chamber, e.g., at a force, e.g., effective force, of at or about 2000 g.

[0036] In some examples, the rotation of the chamber during at least a portion of the incubation is at a force of greater than at or about 200 g, greater than at or about 300 g, or greater than at or about 500 g, such as greater than at or about 1000 g, 1500 g, 2000 g, 2500 g, 3000 g, or 3200 g, at an internal wall of the cavity of the centrifugal chamber and/or a layer, e.g., surface layer, of the cells. In some examples, the force is at least at or about 1000 g, 1500 g, 2000 g, or 2500 g, 3000 g or 3200 g. In some examples, the force is at or about 2100 g, 2200 g or 3000 g.

[0037] In some examples, the methods include incubating an input composition containing cells and viral particles containing a recombinant viral vector, at least a portion of said incubating being carried out under rotating conditions, thereby generating an output composition containing a plurality of the cells transduced with the viral vector, wherein said input composition contains greater than or about 20 mL, 50 mL, at least 100 mL, or at least 150 mL in volume, and/or said input composition comprises at least 1×10^8 cells; and said rotating conditions comprise a relative centrifugal force on a surface layer of the cells of greater than about 1500 g.

[0038] In some examples of the methods, at least 25 % or at least 50 % of said cells in the output composition are transduced with said viral vector; and/or at least 25 % or at least 50 % of said cells in the output composition express a product of a heterologous nucleic acid contained within said viral vector.

[0039] In some of any such examples, said incubation is carried out in a cavity of a centrifugal chamber and the number of said cells in said input composition is at or about the number of said cells sufficient to form a monolayer or a bilayer on the inner surface of said cavity during said rotation.

[0040] In some examples, said centrifugal chamber includes an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, wherein at least a portion of said side wall surrounds said internal cavity and said at least one opening is capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity,

[0041] In some examples, said centrifugal chamber further includes a movable member and said internal cavity is a cavity of variable volume defined by said end wall, said

substantially rigid side wall, and said movable member, said movable member being capable of moving within the chamber to vary the internal volume of the cavity.

[0042] In some of any such examples, the input composition in said cavity contains a liquid volume of at least 20 mL or at least 50 mL and at or about 1 million cells per cm^2 of the internal surface area of the cavity during at least a portion of said incubation.

[0043] In some of any such examples, a further portion of the incubation is carried out outside of the centrifugal chamber and/or without rotation, said further portion carried out subsequent to the at least a portion carried out in the chamber and/or with rotation.

[0044] In some of any such examples, the at least a portion of the incubation carried out in the cavity of the centrifugal chamber and/or the further portion of the incubation is effected at or at about 37 °C ± 2 °C.

[0045] In some of any such examples, the incubation further includes transferring at least a plurality of the cells to a container during said incubation and said further portion of the incubation is effected in the container. In some examples, the transferring is performed within a closed system, wherein the centrifugal chamber and container are integral to the closed system.

[0046] In some of any such examples, the incubation is carried out for a time between at or about 1 hour and at or about 96 hours, between at or about 4 hours and at or about 72 hours, between at or about 8 hours and at or about 48 hours, between at or about 12 hours and at or about 36 hours, between at or about 6 hours and at or about 24 hours, between at or about 36 hours and at or about 96 hours, inclusive; or the further portion of the incubation is carried out for a time between at or about 1 hour and at or about 96 hours, between at or about 4 hours and at or about 72 hours, between at or about 8 hours and at or about 48 hours, between at or about 12 hours and at or about 36 hours, between at or about 6 hours and at or about 24 hours, between at or about 36 hours and at or about 96 hours, inclusive.

[0047] In some of any such examples, the incubation or further portion of the incubation is carried out for a time that is no more than 48 hours, no more than 36 hours or no more than 24 hours; or the further portion of the incubation is carried out for a time that is no more than 48 hours, no more than 36 hours or no more than 24 hours.

[0048] In some of any such examples, the incubation is performed in the presence of a stimulating agent; and/or the further portion of the incubation is performed in the presence of a stimulating agent.

[0049] In some of any such examples, the incubation is carried out for a time that is no more than 24 hours; the cells in the composition have not been subjected to a temperature of greater than 30 °C for more than 24 hours; and/or the incubation is not performed in the presence of a stimulating agent.

[0050] In some of any such examples, the stimulating agent is an agent capable of inducing proliferation of T cells, CD4+ T cells and/or CD8+ T cells.

[0051] In some of any such examples, the stimulating agent is a cytokine selected from among IL-2, IL-15 and IL-7.

[0052] In some of any such examples, the output composition containing transduced cells contains at least 1 x 10^7 cells or at least 5 x 10^7 cells.

[0053] In some of any such examples, the output composition containing transduced cells contains at least 1 x 10^8 cells, 2 x 10^8 cells, 4 x 10^8 cells, 6 x 10^8, 8 x 10^8 cells or 1 x 10^9 cells.

[0054] In some of any such examples, the cells are T cells. In some examples, the T cells are unfractionated T cells, isolated CD4+ T cells and/or isolated CD8+ T cells.

[0055] In some of any such examples, the method results in integration of the viral vector into a host genome of one or more of the at least a plurality of cells and/or into a host genome of at least at or about 20 % or at least at or about 30 % or at least at or about 40 % of the cells in the output composition.

[0056] In some of any such examples, at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said input composition are transduced with said viral vector by the method; and/or at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said output composition are transduced with said viral vector; and/or at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said output composition express a product of a heterologous nucleic acid contained within said viral vector.

[0057] Particular examples include methods of transduction carried out by incubating an input composition comprising cells and viral vector particles under rotating conditions, whereby a plurality of the cells are inoculated for transduction with the viral vector, wherein the input composition includes a total volume greater than 50 mL, such as at least 100 mL, or at least 150 mL in volume, and/or said input composition comprises at least 1 x 10^8 cells; and the rotating

conditions comprise centrifugal force of greater than about 1500 g. In some such examples, the incubation is carried out in a cavity of a centrifugal chamber and the number of said cells in said input composition is at or about the number of said cells sufficient to form a monolayer on the inner surface of the cavity during the rotation. In some such examples, at least 25 % or at least 50 % of said cells are transduced with the viral vector.

[0058] In some examples, the methods result in at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said input composition being transduced with the viral vector, and/or produce an output composition in which at least 10 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of the cells are transduced with the vector and/or express a recombinant product encoded by the vector. In some examples, transduction efficiency is expressed for a particular input amount or relative amount of virus. For example, in some examples, such efficiencies are achieved by the methods for an input composition comprising a virus at a ratio of about 1 or about 2 IU per cells.

[0059] In some examples, among all the cells in said output composition produced by the methods, the average copy number of the recombinant viral vector is no more than about 10, no more than about 5, no more than about 2.5, or no more than about 1.5. In some examples, among the cells in the output composition that contain the recombinant viral vector, the average copy number of the vector is no more than about 5, no more than about 2, no more than about 1.5, or no more than about 1.

[0060] In some of any such examples, among all the cells in said output composition that contain the recombinant viral vector or into which the viral vector is integrated, the average copy number of said recombinant viral vector is no more than about 10, no more than about 5, no more than about 2.5, or no more than about 1.5; or among the cells in the output composition, the average copy number of said vector is no more than about 2, no more than about 1.5, or no more than about 1.

[0061] In some examples, the centrifugal chamber is integral to a closed system, for example, where the closed system includes the chamber and at least one tubing line operably linked to the at least one opening via at least one connector, such that liquid and gas are permitted to move between said cavity and said at least one tubing line in at least one configuration of the system. The at least one tubing line typically includes a series of tubing lines. The at least one connector typically includes a plurality of connectors. The closed system may further include at least one container operably linked to the series of tubing lines, such that

the at least one connection permits liquid and/or gas to pass between the at least one container and the at least one opening via the series of tubing lines.

[0062] The at least one connector may include one or more connectors selected from the group consisting of valves, luer ports, and spikes, e.g., a rotational valve, such as a stopcock or multirotational port, and/or an aseptic connector.

[0063] The at least one container may include one or more bags, vials, and/or syringes, and may include container(s) designated as a diluent container, a waste container, a product collection container, output container, and/or an input container.

[0064] In some examples, the at least one container includes at least one input container including the virus and/or cells (which may be a single input container comprising the virus and cells or two input containers comprising the virus and cells, respectively), a waste container, a product container, and at least one diluent or wash solution-containing container, each connected to said cavity via said series of tubing lines and the at least one opening.

[0065] In some of any such examples, at least one container further includes a container that contains a gas prior to and/or during at least a point during said incubation and/or the closed system further includes a microbial filter capable of taking in gas to the internal cavity of the centrifugal chamber and/or the closed system contains a syringe port for effecting intake of gas.

[0066] The methods in some examplesfurther include, prior to and/or during the incubation, effecting intake of the input composition into said cavity. The intake may include flow of liquid from the at least one input container into the cavity through said at least one opening. The intake may include intake of virus from one input container and input of cells from another, to produce the input composition for incubation.

[0067] In some examples, the method includes, prior to and/or during said incubation, providing or effecting intake of gas into said cavity under sterile conditions, said intake being effected by (a) flow of gas from the container that includes gas, (b) flow of gas from an environment external to the closed system, via the microbial filter, or (c) flow of gas from a syringe connected to the system at the syringe port.

[0068] In some examples, the effecting intake of the gas into the internal cavity of the centrifugal chamber is carried out simultaneously or together with the effecting intake of the input composition to the internal cavity of the centrifugal chamber.

[0069] In some of any such examples, the input composition and gas are combined in a single container under sterile conditions outside of the chamber prior to said intake of said input composition and gas into the internal cavity of the centrifugal chamber.

[0070] In some examples, the effecting of the intake of the gas is carried out separately, either simultaneously or sequentially, from the effecting of the intake of the input composition into said cavity.

[0071] In some of any such examples, the intake of gas is effected by permitting or causing flow of the gas from a sterile closed container containing the gas, an external environment through a microbial filter, or a syringe containing said gas.

[0072] In some of any such examples, the gas is air.

[0073] In some examples of the provided process methods, the incubation is part of a continuous process, where the method further includes, during at least a portion of the incubation, effecting continuous intake of said input composition into the cavity, typically during rotation of the chamber, and during a portion of the incubation, effecting continuous expression (i.e. outtake) of liquid from said cavity through said at least one opening, typically during rotation of the chamber. The continuous intake and outtake in some examples occur simultaneously.

[0074] In some examples, the method includes during a portion of said incubation, effecting continuous intake of gas into said cavity during rotation of the chamber; and/or during a portion of said incubation, effecting continuous expression of gas from said cavity.

[0075] In some examples, the method includes the expression of liquid and the expression of gas from said cavity, where each is expressed, simultaneously or sequentially, into a different container.

[0076] In some of any such examples, at least a portion of the continuous intake and the continuous expression occur simultaneously.

[0077] In some examples, the incubation is part of a semi-continuous process, such as one in which the method further includes effecting intake of the input composition into the cavity through the at least one opening, conducting all or part of the incubation, such as the centrifugation, and then effecting expression of liquid from the cavity, and then repeating the process, whereby another input composition is taken in to the cavity, followed by centrifugation, followed by expression. The process can be iterative and include several more rounds of intake, processing, and expression.

[0078] In some of any such examples, the incubation is part of a semi-continuous process, the method further including prior to said incubation, effecting intake of said input composition, and optionally gas, into said cavity through said at least one opening; subsequent to said incubation, effecting expression of liquid and/or optionally gas from said cavity;

effecting intake of another input composition including cells and said viral particles containing a recombinant viral vector, and optionally gas, into said internal cavity; and incubating said another input composition in said internal cavity, wherein the method generates another output composition containing a plurality of cells of the another input composition that are transduced with said viral vector.

[0079] In some of any such examples, said providing or said intake of the input composition into the cavity includes intake of a single composition including the cells and the viral particles containing the recombinant viral vector; or intake of a composition including the cells and a separate composition containing the viral particles containing the recombinant viral vector, whereby the compositions are mixed, effecting intake of the input composition.

[0080] The intake may include intake of a single composition containing the cells and the virus; or intake of a composition containing the cells and a separate composition containing the virus, whereby the compositions are mixed, effecting intake of the input composition. In some examples of the continuous or semi-continuous process, at least 1 x 10^8 cells or at least 1x10^9 cells or at least 1x10^{10} cells or more are processed in total, over the multiple rounds or continuous process.

[0081] In some examples, the method includes effecting rotation of the centrifugal chamber prior to and/or during said incubation and effecting expression of liquid from the cavity into said waste container following the incubation; effecting expression of liquid from the at least one diluent container into said cavity via the at least one opening and effecting mixing of the contents of the cavity; and effecting expression of liquid from said cavity into the product container, thereby transferring cells transduced with the viral vector into the product bag.

[0082] In some examples, the method further includes carrying out other processing steps, or at least a portion of one or more other processing steps, within the same chamber and/or closed system. In some examples, the one or more processing steps can includes processes in which the cells are isolated, such as separated or selected, stimulated, and formulated within the same chamber and/or closed system. In some cases, the one or more further processing steps also can include washing cells, suspending cells and/or diluting or concentrating cells, which can be carried out prior to or subsequent to any one or more of the processing steps for isolating, such as separating or selecting, stimulating, transducing and/or formulating the cells. In some examples, the one or more other processing steps can be carried out prior to, simultaneously with or subsequent to the incubation of cells with the viral vector particles in the methods of transduction. In some examples, the one or more further processing steps, or a portion of

the one or more further processing steps, can be carried out in a cavity of a centrifugal chamber that is the same or different as a cavity of a centrifugal chamber employed in the incubation of cells with the viral vector particles.

[0083] Among the provided processing methods, including isolation, e.g. selection, methods, stimulation methods, formulation methods and other processing methods, are those carried out according to any of the examples as described above.

[0084] For example, in some examples, the method further includes (a) washing a biological sample (e.g., a whole blood sample, a buffy coat sample, a peripheral blood mononuclear cells (PBMC) sample, an unfractionated T cell sample, a lymphocyte sample, a white blood cell sample, an apheresis product, or a leukapheresis product) containing cells in a cavity of a chamber, prior to the incubation for isolating, e.g. selecting cells, and/or prior to the incubation for incubating cells with viral vector particles, (b) isolating, e.g. selecting, the cells from a sample (e.g., a whole blood sample, a buffy coat sample, a peripheral blood mononuclear cells (PBMC) sample, an unfractionated T cell sample, a lymphocyte sample, a white blood cell sample, an apheresis product, or a leukapheresis product) in a cavity prior to the incubation of such cells with viral vector particles and/or (c) stimulating cells in a cavity prior to and/or during the incubation of such cells with viral vector particles, e.g., by exposing cells to stimulating conditions, thereby inducing cells of the input composition to proliferate. In some examples, the isolating includes immunoaffinity-based selection.

[0085] In some of any such examples, the method includes (a) washing a biological sample containing said cells in an internal cavity of a centrifugal chamber prior to said incubation; and/or (b) isolating said cells from a biological sample, wherein at least a portion of the isolation step is performed in an internal cavity of a centrifugal chamber prior to said incubation; and/or (c) stimulating cells prior to and/or during said incubation, said stimulating including exposing said cells to stimulating conditions, thereby inducing cells of the input composition to proliferate, wherein at least a portion of the step of stimulating cells is performed in an internal cavity of a centrifugal chamber.

[0086] In some examples, the methods may further include isolation, e.g., selection, of the cells in the chamber, e.g., by immunoaffinity based selection. In some examples, the isolation, e.g. selection, of cells is carried out prior to the incubation of cells with the viral vector particles in the methods of transduction, whereby the isolated, such as selected, cells are the cells present in the input composition and/or incubated with the viral vector particles. In some examples, the isolation, e.g., selection, includes incubation of cells with a selection reagent,

such as an immunoaffinity reagent. In some examples, at least a portion of the isolation, e.g. selection, step, such as incubation of cells with a selection reagent, e.g. an immunoaffinity reagent, is carried out in the cavity of a chamber, which, in some cases, can include rotation of the chamber, for example, for mixing of the reagent and cells.

[0087] In some examples, the methods may further include stimulating cells prior to, during and/or subsequent to the incubation of cells with the viral vector particles, in which at least all or a portion of the stimulation can be carried out in a cavity of a centrifugal chamber. In some examples, the stimulating conditions may include incubation of cells in the presence of an agent capable of activating one or more intracellular signaling domains of one or more components of a TCR complex, such as a primary agent that specifically binds to a member of a TCR complex, e.g., CD3, and a secondary agent that specifically binds to a T cell costimulatory molecule, e.g., CD28, CD137 (4-1-BB), OX40, or ICOS, including antibodies such as those present on the surface of a solid support, such as a bead. In some examples, at least a portion of the stimulation, such as incubation of cells in the presence of a stimulating condition, is carried out in the cavity of a chamber, which, in some cases, can include rotation of the chamber, for example, for mixing of the reagent and cells.

[0088] In some of any such examples, the method includes formulating cells, such as cells produced or generated in accord with the provided methods, including cell transduced by the method, in a pharmaceutically acceptable buffer in an internal cavity of a centrifugal chamber, thereby producing a formulated composition. In some examples, the methods further include effecting expression of the formulated composition to one or a plurality of containers. In some examples, the methods include the effecting of expression of the formulated composition includes effecting expression of a number of the cells present in a single unit dose to one or each of said one or a plurality of containers.

[0089] In some of any such examples, each of said a cavity of a centrifugal chamber is the same or different as a cavity of a centrifugal employed in one or more of the other steps and/or in the process of incubating and/or rotating an input composition containing cells and viral particles.

[0090] In some of any such examples, each of said centrifugal chambers is integral to a closed system, said closed system including said chamber and at least one tubing line operably linked to the at least one opening via at least one connector, whereby liquid and gas are permitted to move between said cavity and said at least one tubing line in at least one configuration of said system.

[0091] The cells processed by the methods typically are primary cells, such as cells obtained from a subject, typically a human. The cells may be derived from a subject to which the therapy is to be administered, such as one having a disease or condition targeted by a recombinant molecule expressed by a vector transduced, e.g., a recombinant antigen receptor such as a chimeric antigen receptor or transgenic TCR. Alternatively, the cells may be from a different subject. Thus, the methods encompass processing for autologous and allogeneic transfer. The cells may include suspension cells, e.g., white blood cells, e.g., T cells, such as isolated $CD8^+$ T cells, or isolated $CD4^+$ T cells or subsets thereof, or NK cells.

[0092] In some examples, during the incubation, the centrifugal chamber is associated with a sensor, for example, a sensor capable of monitoring the position of the movable member, and control circuitry, such as circuitry capable of receiving and transmitting information to and from the sensor, causing movement of said movable member, and/or that is further associated with a centrifuge and thus is capable of causing rotation of the chamber during said incubation.

[0093] In some examples, the chamber contains the movable member and during the incubation is located within a centrifuge and associated with a sensor capable of monitoring the position of the movable member, and control circuitry capable of receiving and transmitting information from the sensor and causing movement of the movable member, intake and expression of liquid to and from said cavity via said one or more tubing lines, and rotation of the chamber via the centrifuge.

[0094] In some examples, the chamber, control circuitry, centrifuge, and/or sensor are housed within a cabinet, e.g., during the incubation.

[0095] In some examples of any of the viral transfer, e.g., transduction methods, the recombinant viral vector encodes a recombinant receptor, which is thereby expressed by cells of the output composition. In some examples, the recombinant receptor is a recombinant antigen receptor, such as a functional non-T cell receptor, e.g., a chimeric antigen receptor (CAR), or a transgenic T cell receptor (TCR). In some examples, the recombinant receptor is a chimeric receptor containing an extracellular portion that specifically binds to a ligand and an intracellular signaling portion containing an activating domain and a costimulatory domain.

[0096] In some of any such examples, the cells include primary human T cells obtained from a human subject and prior to the incubation with viral vector particles and/or prior to completion of the transduction and/or, where the method includes formulation, prior to the formulation, the primary human T cells have not been present externally to the subject at a temperature of greater than 30°C for greater than 1 hour, greater than 6 hours, greater than 24

hours, or greater than 48 hours or prior to the incubation and/or prior to the completion of the transduction, and/or where the method includes formulation, prior to the formulation, the primary human T cells have not been incubated in the presence of an antibody specific for CD3 and/or an antibody specific for CD28 and/or a cytokine, for greater than 1 hour, greater than 6 hours, greater than 24 hours, or greater than 48 hours.

[0097] Provided herein are methods for isolation, e.g. selection, of cells including (a) incubating a selection reagent and primary cells in an internal cavity of a centrifugal chamber under mixing conditions, whereby a plurality of the primary cells bind to said selection reagent and (b) separating the plurality of the primary cells from another one or more of the primary cells based on binding to the selection reagent, thereby enriching the primary cells based on binding to the selection reagent, wherein the centrifugal chamber is rotatable around an axis of rotation and the internal cavity has a maximum volume of at least 50 mL, at least 100 mL, or at least 200 mL. In some examples, the methods for isolation, e.g. selection, occur in a closed system. In some examples, prior to the step of separating the plurality of cells, the cells incubated with the selection reagent, are expressed from or transferred out of the chamber, but maintained in the closed system. In some examples, optionally, subsequent to incubation with the selection reagent and prior to separating the cells, the method further includes one or more washing steps, which in some cases, can be performed in the cavity of the chamber in accord with the provided methods. In some examples, the step of separating the cells can be effecting using a solid support, such as using an immunoaffinity-column, including those for magnetic separation, which can be contained in the closed system.

[0098] Provided herein are methods for stimulation of cells, including incubating a stimulation agent and primary cells under conditions whereby the stimulation agent binds to a molecule expressed by a plurality of the primary cells and the plurality of the cells are activated or stimulated, wherein at least a portion of the incubation is carried out in an internal cavity of a centrifugal chamber under mixing conditions, where the centrifugal chamber is rotatable around an axis of rotation and the internal cavity has a maximum volume of at least 50 mL, at least 100 mL, or at least 200 mL.

[0099] In some examples, the methods of stimulation are performed as part of a process that includes transducing cells, whereby all or a part of such process is performed in a centrifugal chamber and/or as part of the same closed system. In some examples, the primary cells that are stimulated with a stimulation agent include or are cells obtained following isolation, e.g. selection, of cells from a biological sample, such as in accord with the provided

methods. In some examples, at least a portion of the stimulation is carried out simultaneously or during the incubation of cells with the viral vector particles, such that the primary cells include or are cells present in the input composition and/or are cells in which transduction has occurred or is initiated. In some examples, at least a portion of the stimulation is carried out prior to the incubation of cells with the viral vector particles, such that the cells incubated with the viral vector particles are stimulated cells, which, in some cases, includes proliferating cells.

[0100] In some examples, at least a portion of the one of more other processing steps of the method, including isolation, e.g. selection, stimulation, washing and/or formulation, that is carried out in a chamber includes where the chamber includes an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, wherein at least a portion of the side wall surrounds the internal cavity and the at least one opening is capable of permitting intake of liquid into the internal cavity and expression of liquid from the cavity.

[0101] Provided herein are compositions containing transduced cells produced by the methods of any of the above examples. In some of any such examples, the composition contains cells that are primary cells and/or human cells and/or include white blood cells, and/or T cells, and/or NK cells. In some of any such examples, the composition contains at least 5 x 10^7 cells, 1 x 10^8 cells, 2 x 10^8 cells, 4 x 10^8 cells, 6 x 10^8 cells, 8 x 10^8 cells or 1 x 10^9 cells. In some of any such examples, the composition contains a therapeutically effective number of cells for use in adoptive T cell therapy. In some of any such examples, the cells are T cells and subsequent to transduction, the cells in the composition are not subjected to cell expansion in the presence of a stimulating agent and/or the cells are not incubated at a temperature greater than 30 °C for more than 24 hours or the composition does not contain a cytokine or the composition does not contain a stimulating agent that specifically binds to CD3 or a TCR complex.

[0102] Provided herein are compositions containing at least 1 x 10^7 or at least 5 x 10^7 T cells, at least a plurality of which are transduced with a recombinant viral vector, where subsequent to transduction, the cells in the composition have not been subjected to cell expansion in the presence of a stimulating agent and/or the cells have not been incubated at a temperature greater than 30 °C for more than 24 hours and/or at least 30, 40, 50, 60, 70, or 80 % of the T cells in the composition contain high surface expression of CD69 or TGF-beta-II. In some examples, the composition contains at least 1 x 10^8 cells, 2 x 10^8 cells, 4 x 10^8 cells, 6 x 10^8, 8 x 10^8 cells or 1 x 10^9 cells.

[0103] In some of any such examples, the T cells are unfractionated T cells, isolated CD8+ T cells, or isolated CD4+ T cells.

[0104] In some of any such examples, at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said composition are transduced with the viral vector.

[0105] In some of any such examples, the viral vector encodes a recombinant receptor and transduced cells in the composition express the recombinant receptor. In some examples, the recombinant receptor is a recombinant antigen receptor. In some examples, the recombinant antigen receptor is a functional non-T cell receptor. In some examples, the functional non-T cell receptor is a chimeric antigen receptor (CAR). In some examples, the recombinant receptor is a chimeric receptor containing an extracellular portion that specifically binds to a ligand and an intracellular signaling portion containing an activating domain and a costimulatory domain. In some examples, the recombinant antigen receptor is a transgenic T cell receptor (TCR).

[0106] In some of any such examples, among all the cells in the composition, the average copy number of the recombinant viral vector is no more than about 10, no more than 8, no more than 6, no more than 4, or no more than about 2, or among the cells in the composition transduced with the recombinant viral vector, the average copy number of said vector is no more than about 10, no more than 8, no more than 6, no more than 4, or no more than about 2.

[0107] In some of any such examples, the composition contains a pharmaceutically acceptable excipient.

[0108] Provided herein are methods of treatment, including administering to a subject having a disease or condition the composition of any of the above examples. In some examples, the transduced T cells in the composition exhibit increased or longer expansion and/or persistence in the subject than transduced T cells in a composition in which, subsequent to transduction, the cells in the composition have been subjected to cell expansion in the presence of a stimulating agent and/or the cells have been incubated at a temperature greater than 30 °C for more than 24 hours.

[0109] In some of any such examples, the recombinant receptor, chimeric antigen receptor or transgenic TCR specifically binds to an antigen associated with the disease or condition. In some examples, the disease or condition is a cancer, an autoimmune disease or disorder, or an infectious disease.

[0110] Provided herein are compositions containing at least 1 x 10^7 cells and at least at or about 1 infectious unit (IU) per cell of viral particles containing a recombinant viral vector. In some examples, the cells contain at least or about 50 x 10^6 cells, 100 x 10^6 cells, or 200 x 10^6 cells, and/or said viral particles are present in the composition in an amount that is at least 1.6 IU/cell, 1.8 IU/cell, 2.0 IU/cell, 2.4 IU/cell, 2.8 IU/cell, 3.2 IU/cell, 3.6 IU/cell, 4.0 IU/cell, 5.0 IU/cell, 6.0 IU/cell, 7.0 IU/cell, 8.0 IU/cell, 9.0 IU/cell or 10.0 IU/cell.

[0111] In any of such examples, the liquid volume of the composition is less than or equal to 220 mL, less than or equal to 200 mL, less than or equal to 100 mL, less than or equal to 50 mL or less than or equal to 20 mL.

[0112] In some of any such examples, the cells are primary cells. In some of any such examples, the cells are human cells. In some of any such examples, the cells include suspension cells, the cells include white blood cells and/or the cells include T cells or NK cells. In some examples, the cells are T cells and the T cells are unfractionated T cells, isolated CD8+ T cells, or isolated CD4+ T cells.

[0113] In some of any such examples, the viral vector encodes a recombinant receptor. In some examples, the recombinant receptor is a recombinant antigen receptor. In some examples, the recombinant antigen receptor is a functional non-T cell receptor. In some examples, the functional non-T cell receptor is a chimeric antigen receptor (CAR). In some examples, the recombinant receptor is a chimeric receptor containing an extracellular portion that specifically binds to a ligand and an intracellular signaling portion containing an activating domain and a costimulatory domain. In some examples, the recombinant antigen receptor is a transgenic T cell receptor (TCR).

[0114] Provided herein are centrifugal chambers rotatable around an axis of rotation, including an internal cavity containing the composition of any of the above examples.

[0115] Provided herein are centrifugal chambers rotatable around an axis of rotation, containing an internal cavity containing (a) a composition containing at least 5 x 10^7 primary T cells transduced with a recombinant viral vector and/or (b) a composition containing at least 5 x 10^7 primary T cells and viral particles containing a recombinant viral vector.

[0116] In some of any such examples, the chamber further contains an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, wherein at least a portion of said side wall surrounds said internal cavity and said at least one opening is capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity.

[0117] In some of any such examples, said composition in said cavity contains at least 1 x 10^8 cells, 2 x 10^8 cells, 4 x 10^8 cells, 6 x 10^8 cells, 8 x 10^8 cells or 1 x 10^9 of the cells.

[0118] In some of any such examples, the T cells are unfractionated T cells, isolated CD8+ T cells, or isolated CD4+ T cells.

[0119] In some of any such examples of the chamber, at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said composition are transduced with a viral vector.

[0120] In some of any such examples of the chamber, the viral vector encodes a recombinant receptor and cells in the composition express the recombinant receptor. In some examples, the recombinant receptor is a recombinant antigen receptor. In some examples, the recombinant antigen receptor is a functional non-T cell receptor. In some examples, the functional non-T cell receptor is a chimeric antigen receptor (CAR). In some examples, the recombinant receptor is a chimeric receptor containing an extracellular portion that specifically binds to a ligand and an intracellular signaling portion containing an activating domain and a costimulatory domain. In some examples, the recombinant antigen receptor is a transgenic T cell receptor (TCR).

[0121] In some of any such examples of the chamber, among all the cells in the composition, the average copy number of said recombinant viral vector is no more than about 10, no more than 8, no more than 6, no more than 4, or no more than about 2 or among the cells in the composition transduced with the recombinant viral vector, the average copy number of said vector is no more than about 10, no more than 8, no more than 6, no more than 4, or no more than about 2.

[0122] Provided herein are centrifugal chambers rotatable around an axis of rotation, including an internal cavity containing the composition of any of the above examples. In some examples, the chamber further contains a volume of gas up to the maximum volume of the internal cavity of the chamber. In some examples, the gas is air.

[0123] In some of any such examples of the chamber, the chamber is rotatable around an axis of rotation and includes an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, wherein at least a portion of said side wall surrounds said internal cavity and said at least one opening is capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity. In some examples the side wall is curvilinear. In some examples the side wall is generally cylindrical.

[0124] In some of any such examples of the chamber, said at least one opening includes an inlet and an outlet, respectively capable of permitting said intake and expression or said at least one opening includes a single inlet/outlet, capable of permitting said intake and said expression. In some of any such examples of the chamber, aid at least one opening is coaxial with the chamber and is located in the end wall.

[0125] In some of any such examples, the chamber further includes a movable member and said internal cavity is a cavity of variable volume defined by said end wall, said substantially rigid side wall, and said movable member, said movable member being capable of moving within the chamber to vary the internal volume of the cavity. In some examples, the movable member is a piston and/or the movable member is capable of axially moving within the chamber to vary the internal volume of the cavity.

[0126] In some of any such examples, the internal surface area of said cavity is at least at or about 1 x 10^9 μm^2, the internal surface area of said cavity is at least at or about 1 x 10^{10} μm^2, the length of said rigid wall in the direction extending from said end wall is at least about 5 cm, the length of said rigid wall in the direction extending from said end wall is at least about 8 cm and/or the cavity contains a radius of at least about 2 cm at at least one cross-section.

[0127] In some of any such examples of the chamber, the liquid volume of said composition present in said cavity is between or between about 0.5 mL per square inch of the internal surface area of the cavity (mL/sq.in) and 5 mL/sq.in, 0.5 mL/sq.in. and 2.5 mL/sq.in., 0.5 mL/sq.in. and 1 mL/sq.in., 1 mL/sq.in. and 5 mL/sq.in., 1 mL/sq.in. and 2.5 mL/sq.in. or 2.5 mL/sq.in. and 5 mL/sq.in. In some of any such examples, the liquid volume of said composition present in said cavity is at least 0.5 mL/sq.in., 1 mL/sq.in., 2.5 mL/sq.in., or 5 mL/sq.in.

[0128] Provided herein are closed systems containing the centrifugal chamber of any of the above examples. In some of any such examples of the closed system, the centrifugal chamber is capable of rotation at a speed up to 8000 g, wherein the centrifugal chamber is capable of withstanding a force of 500, 1000, 1500, 2000, 2500, 3000 or 3200 g, without substantially yielding, bending, or breaking or otherwise resulting in damage of the chamber and/or while substantially holding a generally cylindrical shape under such force.

Brief Description of the Drawings

[0129] **FIG. 1A** shows transduction efficiency calculated as percentage of $CD3^+$ T cells with surface expression of a chimeric antigen receptor (CAR) encoded by a viral vector, following

CLAIMS

1. A transduction method, comprising incubating, in an internal cavity of a centrifugal chamber, an input composition comprising cells and viral particles containing a recombinant viral vector, wherein

said centrifugal chamber comprises:

an end wall, a substantially rigid side wall extending from said end wall, and one or more opening, wherein at least a portion of said side wall surrounds said internal cavity and at least one of the one or more opening is capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity;

a movable member capable of moving within the chamber to vary the internal volume of the cavity, whereby the internal cavity is a cavity of variable volume defined by said end wall, said substantially rigid side wall, and said movable member;

the centrifugal chamber is rotatable around an axis of rotation and is rotating around said axis of rotation during at least a portion of the incubation; and

the method generates an output composition comprising a plurality of the cells transduced with the viral vector.

2. The method of claim 1, wherein said rotating comprises rotation at a relative centrifugal force at an internal surface of the side wall of the cavity and/or at a surface layer of the cells that is at least at or about 600 g, 1000 g, or 1600 g.

3. The method of claim 1 or 2, wherein the at least a portion of the incubation during which the chamber is rotating is for a time that is:

greater than or about 10 minutes, greater than or about 30 minutes, or greater than or about 60 minutes or

between or between about 5 minutes and 60 minutes.

4. The method of any of claims 1-3, wherein:

the average liquid volume of said input composition present in said cavity during said incubation is no more than about 5 milliliters (mL) per square inch of the internal surface area of the cavity during said incubation;

the maximum liquid volume of said input composition present in said cavity at any one time during said incubation is no more than about 5 mL per square inch of the maximum internal surface area of the cavity;

the maximum liquid volume of said input composition present in said cavity at any one time during said incubation or the average volume over the course of the incubation is no more than at or about 2 times the volume of a monolayer of said cells formed on the inner surface of said cavity during rotation of said chamber at a force of at or about 1000 g or at or about 2000 g at an internal surface of the side wall and/or at a surface layer of the cells; or

the liquid volume of the input composition is no more than 20 mL, no more than 40 mL, or no more than 100 mL or no more than 200 mL.

5. The method of any of claims 1-4, wherein:

the number of said cells in said input composition is at or about the number of said cells sufficient to form a monolayer on the surface of said cavity during rotation of said centrifugal chamber at a force of at or about 1000 g or at or about 2000 g at an internal surface of the side wall and/or at a surface layer of the cells; and/or

the number of said cells in said input composition is no more than 1.5 times or 2 times the number of said cells sufficient to form a monolayer on the surface of said cavity during rotation of said centrifugal chamber at a force of at or about 1000 g or at or about 2000 g at an internal surface of the side wall and/or at a surface layer of the cells; and/or

said input composition in the cavity comprises at least at or about 1×10^6 of said cells, 5×10^6 of said cells, 1×10^7 of said cells or 1×10^8 of said cells.

6. The method of any of claims 1-5, wherein

said input composition comprises at least at or about 1 infectious unit (IU) of viral particles per one of said cells.

7. The method of any of claims 1-6, wherein, during at least a portion of the incubation in the chamber or during the rotation of the chamber, the liquid volume of the input composition occupies only a portion of the volume of the internal cavity of the chamber, the volume of the cavity during said at least a portion or during said rotation further comprising a

gas, said gas taken into said cavity via at least one of the one or more opening, prior to or during said incubation.

8. The method of claim 7, wherein the method further comprises, prior to or during said incubation, effecting movement of the movable member, thereby increasing the internal volume of the internal cavity and effecting the intake of the gas into the internal cavity.

9. The method of any of claims 1-8, wherein:

the movable member is a piston; and/or

the movable member is capable of axially moving within the chamber to vary the internal volume of the cavity.

10. The method of any of claims 1-9, wherein a further portion of the incubation is carried out outside of the centrifugal chamber and/or without rotation, said further portion carried out subsequent to the at least a portion carried out in the chamber and/or with rotation.

11. The method of claim 10, wherein:

the incubation and/or the further portion of the incubation is carried out for a time that is no more than 24 hours;

the cells in the composition have not been subjected to a temperature of greater than 30 °C for more than 24 hours; and/or

the incubation and/or the further portion of the incubation is not performed in the presence of a stimulating agent.

12. The method of any of claims 1-11, wherein:

the output composition containing transduced cells comprises at least at or about 1×10^7 cells or at least at or about 5×10^7 cells;

for an input composition comprising a virus at a ratio of about 1 or about 2 IU per cells, said method is capable of producing an output composition in which at least 10 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of the cells in said output composition generated by the method comprise said recombinant viral vector and/or express a product of a recombinant nucleic acid comprised within said vector; or

among all the cells in said output composition that contain the recombinant viral vector or into which the viral vector is integrated, the average copy number of said recombinant viral vector is no more than about 10, no more than about 5, no more than about 2.5, or no more than about 1.5; or among the cells in the output composition, the average copy number of said vector is no more than about 2, no more than about 1.5, or no more than about 1.

13. The method of any of claims 1-12, wherein the centrifugal chamber is integral to a closed system, said closed system comprising:

said chamber and at least one tubing line operably linked to the one or more opening via at least one connector, whereby liquid and gas are permitted to move between said cavity and said at least one tubing line in at least one configuration of said system.

14. The method of claim 13, wherein the closed system comprises at least one container operably linked to the at least one tubing line, the connection permitting liquid and/or gas to pass between said at least one container and said at least one opening via the at least one tubing line.

15. The method of claim 13 or 14, wherein said at least one container comprises at least one input container comprising said viral vector particles and said cells, a waste container, a product container, and at least one diluent container, each connected to said cavity via said at least one tubing line and said one or more opening.

16. The method of claim 15 wherein said method further comprises:

prior to and/or during said incubation, effecting intake of said input composition into said cavity, said intake comprising flowing of liquid from said at least one input container into said cavity through said at least one opening; and/or

prior to and/or during said incubation, providing or effecting intake of gas into said cavity under sterile conditions, said intake being effected by (a) flow of gas from a container that comprises gas, (b) flow of gas from an environment external to the closed system, via a microbial filter, or (c) flow of gas from a syringe connected to the system at a syringe port.

17. The method of claim 15 or 16, wherein the closed system comprises:

at least one further container that comprises a gas for intake into said cavity prior to and/or during at least a portion of said incubation; and/or a microbial filter capable of taking in gas to the internal cavity of the centrifugal chamber; and/or a syringe port for effecting intake of gas.

18. The method of claim 16 or 17, wherein:

the effecting intake of the gas into the internal cavity of the centrifugal chamber is carried out simultaneously or together with the effecting intake of the input composition to the internal cavity of the centrifugal chamber; or

the effecting of the intake of the gas is carried out separately, either simultaneously or sequentially, from the effecting of the intake of the input composition into said cavity.

19. The method of any of claims 7-18, wherein the gas is air.

20. The method of any of claims 1-19, wherein the incubation is part of a continuous process, the method further comprising:

during at least a portion of said incubation, effecting continuous intake of said input composition into said cavity during rotation of the chamber; and

during a portion of said incubation, effecting continuous expression of liquid from said cavity through said at least one opening during rotation of the chamber.

21. The method of any of claims 1-19, wherein the incubation is part of a semi-continuous process, the method further comprising:

prior to said incubation, effecting intake of said input composition into said cavity through said at least one opening;

subsequent to said incubation, effecting expression of liquid from said cavity;

effecting intake of another input composition comprising cells and said viral particles containing a recombinant viral vector into said internal cavity; and

incubating said another input composition in said internal cavity,

wherein the method generates another output composition comprising a plurality of cells of the another input composition that are transduced with said viral vector.

22. The method of any of claims 1-19, wherein the incubation is part of a semi-continuous process, the method further comprising:

prior to said incubation, effecting intake of said input composition, and gas, into said cavity through said at least one opening;

subsequent to said incubation, effecting expression of liquid and gas from said cavity;

effecting intake of another input composition comprising cells and said viral particles containing a recombinant viral vector, and gas, into said internal cavity; and

incubating said another input composition in said internal cavity,

wherein the method generates another output composition comprising a plurality of cells of the another input composition that are transduced with said viral vector.

23. The method of any of claims 16-22, wherein the method further comprises:

effecting rotation of said centrifugal chamber prior to and/or during said incubation;

effecting expression of liquid from said cavity into said waste container following said incubation;

effecting expression of liquid from said at least one diluent container into said cavity via said at least one opening and effecting mixing of the contents of said cavity; and

effecting expression of liquid from said cavity into said product container, thereby transferring cells transduced with the viral vector into said product container.

24. The method of any of claims 1-23, further comprising:

(a) washing a biological sample comprising said cells in an internal cavity of a centrifugal chamber prior to said incubation; and/or

(b) isolating said cells from a biological sample, wherein at least a portion of the isolation step is performed in an internal cavity of a centrifugal chamber prior to said incubation; and/or

(c) stimulating cells prior to and/or during said incubation, said stimulating comprising exposing said cells to stimulating conditions, thereby inducing activation and/or proliferation of cells of the input composition, wherein at least a portion of the step of stimulating cells is performed in an internal cavity of a centrifugal chamber.

25. The method of any of claims 1-24, further comprising formulating cells transduced by the method in a pharmaceutically acceptable buffer in an internal cavity of a centrifugal chamber, thereby producing a formulated composition.

26. The method of claim 25, further comprising effecting expression of the formulated composition to one or a plurality of containers.

27. The method of any of claims 1-26, wherein:
said cells in said input composition comprise suspension cells;
said cells in said input composition comprise white blood cells; and/or
said cells in said input composition comprise T cells or NK cells.

28. The method of any of claims 1-27, wherein said cells in said input composition comprise T cells.

29. The method of any of claims 1-28, wherein said cells in said input composition are unfractionated T cells, isolated $CD8^+$ T cells, or isolated $CD4^+$ T cells.

30. The method of any of claims 1-29, wherein said cells in said input composition are human cells.

31. The method of any of claims 1-30, wherein said chamber comprises said movable member and during said incubation, said centrifugal chamber is located within a centrifuge and associated with a sensor, said sensor capable of monitoring the position of said movable member, and control circuitry capable of receiving and transmitting information from said sensor and causing movement of said movable member, intake and expression of liquid and/or gas to and from said cavity via said one or more openings, and rotation of said chamber via said centrifuge.

32. The method of any of claims 1-31, wherein said recombinant viral vector encodes a recombinant receptor, which is thereby expressed by cells of the output composition.

33. The method of claim 32, wherein the recombinant receptor is a chimeric antigen receptor (CAR).

34. The method of claim 32, wherein the recombinant receptor is a transgenic T cell receptor (TCR).