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(54) **Title:** ALL-IN-ONE BIOPROCESS VESSEL FOR ACTIVATION AND EXPANSION OF T-CELLS

(57) **Abstract:** A bioprocess vessel for engineering, activating and expanding T-cells and a method of using such bioprocess vessel is provided. The bioprocess includes a vessel body, a retractable impeller assembly, and an adjustment knob structure. The configuration of the bioprocess vessel solves the problem of T-cell activation in spinner flasks, reducing the time, cost, and contamination risks associated with current spinner flask technologies.

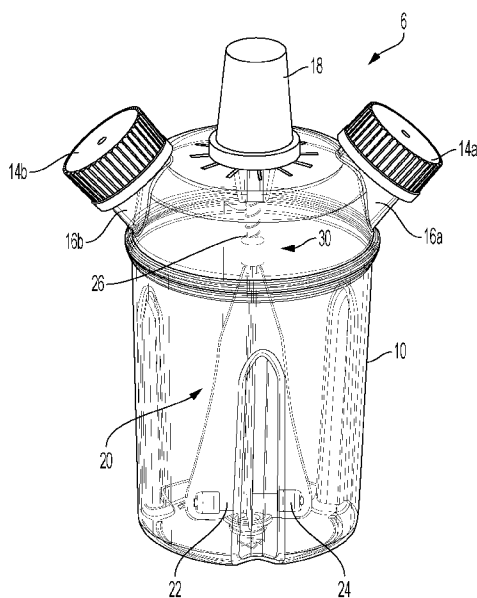


FIG. 2

ALL-IN-ONE BIOPROCESS VESSEL FOR ACTIVATION AND EXPANSION OF T-CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority under 35 U.S.C. §119 of U.S. Provisional Application Serial No. 63/600,969 filed on November 20, 2023, the content of which is relied upon and incorporated herein by reference in its entirety.

FIELD

[0002] The present disclosure relates to the field of bioprocess, and more specifically, to a bioprocess vessel for producing T-cells, including engineered T-cell immunotherapies from patient-derived donor cells, such as chimeric antigen receptor-modified T-cells and other engineered T-cell receptor therapy cells.

BACKGROUND

[0003] Bioprocess is a term used to broadly describe the upstream and downstream processes associated with the production of therapeutic products of interest from cultured cells. One area of interest is engineered cell immunotherapies that use a patient's own T-cells to create a tailored therapy. In particular, chimeric antigen receptor modified T-cells (CAR-T cells) and engineered T-cell receptor (TCR-T cells) immunotherapies have shown remarkable success in the treatment of some hematological malignancies, with potential to treat other cancer types. The production of engineered cell therapies is complex and extremely expensive, making these treatments cost patients as much as \$350K-\$450K dollars for a single treatment. Engineered cell therapies start by extracting a patient's blood and purifying it to obtain T-cells having certain markers. The extracted T-cells are then engineered to produce certain proteins on the outside of the T-cell in the form of a membrane receptor, which helps recognize and assist in destroying targeted malignant cells. In CAR-T cell therapies, which target certain types of blood cancers, a Chimeric Antigen Receptor (CAR) gene is incorporated into the activated T-cells. In TCR-T cell therapies, which target certain types of solid tumors, specific cancer-antigen specific T-cell receptors (TCRs) can be incorporated into the activated T-cells. Once the engineered T-cells have been created (CAR-T cells or TCR-T cells), they are then activated by exposing the T-cells to

certain types of antibodies that are conjugated to a colloidal polymeric nanomatrix or to paramagnetic beads. These engineered T-cells must then be multiplied to a therapeutic level, a phase called expansion. Finally, these multiplied CAR-T cells are then administered back to the patient as a therapeutic.

[0004] Platforms exist to manufacture these cells, ranging from highly manual to fully automated. However, currently existing platforms used for engineered cell therapies are open systems, meaning they require handling and multiple transfer steps throughout the process. The transferring and handling of the cells in current platforms greatly increases contamination risks for each batch and makes it difficult to automate the manufacturing process. This is a major drawback to present platforms as these shortfalls increase the complexity and cost of engineered cell therapies.

[0005] Highly automated, closed, all-in-one systems could provide minimal manipulation but at a significant equipment cost that is typically associated with such devices. There is a need for closed systems to assist in the research and development of engineered cell therapies that are both economical and that minimize the handling and multiple transfer steps that plague the current systems. The present disclosure provides an economical closed system solution that minimizes the handling and transfer steps.

SUMMARY

[0006] According to some aspects of the present disclosure, a bioprocess device is provided that comprises a vessel body, one or more necked access ports, an impeller assembly, a rod, and an adjustment knob structure. The vessel body comprises a top portion and a bottom portion. The rod comprises a first end and a second end. The impeller assembly comprises a shaft and a plurality of planar blades extending from the shaft. The shaft of the impeller assembly is connected to the first end of the rod and the rod extends through the adjustment knob structure. The second end of the rod is connected to a screw external to the adjustment knob structure. Further, the impeller assembly is partially retractable. This bioprocess vessel is configured for activating and expanding T-cells.

[0007] In some embodiments, the second end of the rod comprises a series of spiral grooves that connects to internal spiral grooves on the screw. In some embodiments, the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-sixth and about two-thirds of the height of the vessel body. In other embodiments, the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-third and about one half of the height of the vessel body. In some embodiments, a retracted impeller assembly remains retracted when the screw is released.

[0008] In some embodiments, the impeller assembly comprises a magnet. In some embodiments, no magnetic interference occurs with magnetic beads when the impeller assembly is in a maximumly retracted state.

[0009] In some embodiments, a first o-ring is connected to the first end of the rod and the first end of the rod is inside an inner channel of the shaft of the impeller assembly. In some embodiments, the impeller assembly is freely rotatable against the rod.

[0010] According to some aspects of the present disclosure, a bioprocess vessel is provided that comprises a vessel body comprising a top portion and a bottom portion, one or more necked access ports, an impeller assembly comprising a shaft and a plurality of planar blades extending from the shaft, a rod comprising a first end and a second end, and an adjustment knob structure. Here, the impeller assembly shaft is connected to the first end of the rod, the rod extends through the adjustment knob structure, and the second end of the rod is connected to a first o-ring that is external to the adjustment knob structure. The impeller assembly is partially retractable and the bioprocess vessel is configured for activating and expanding T-cells.

[0011] In some embodiments, the adjustment knob structure comprises a series of internal spiral grooves on its inner surface that connects to a screw comprising a series of spiral grooves on its external surface, and the rod extends through the screw. In some embodiments, the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-sixth and about two-thirds of the height of the vessel body. In other embodiments, the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-third and about one half of the height of the vessel body. In some embodiments, a retracted impeller assembly remains retracted when the adjustment knob structure is released.

[0012] In some embodiments, the impeller assembly comprises a magnet. In some embodiments, no magnetic interference occurs with magnetic beads when the impeller assembly is in a maximumly retracted state.

[0013] In some embodiments, a first o-ring is connected to the first end of the rod and the first end of the rod is inside an inner channel of the shaft of the impeller assembly. In some embodiments, the impeller assembly is freely rotatable against the rod.

[0014] According to some aspects of the present disclosure, a bioprocess vessel is provided that comprises a vessel body comprising a top portion and a bottom portion, one or more necked access ports, an impeller assembly comprising a shaft and a plurality of planar blades extending from the shaft, a rod comprising a first end and a second end, and an adjustment knob structure. In this aspect, the shaft of the impeller assembly is connected to the first end of the rod, the second end of the rod extends through the top portion of the vessel body and through a first screw connected to an external side of the top portion of the vessel body, and the second end of the rod connects to the interior surface of a second screw comprising a series of spiral grooves on its exterior surface. The second screw further comprises an exterior surface that reversibly connects to an interior surface of the adjustment knob structure, and the second screw is connected to a spring that is also connected to the adjustment knob structure. Further, the impeller assembly is partially retractable and the bioprocess vessel is configured for activating and expanding T-cells.

[0015] In some embodiments, a flexible bag-like structure is connected the top portion of the bioprocess vessel by the first screw and to the interior surface of the adjustable knob structure, and the rod and the spring and the second screw are inside the flexible bag-like structure. In some embodiments, the impeller assembly is retracted by connecting the second screw to the first screw and the impeller assembly is extended by disconnecting the second screw from the first screw. In some embodiments, the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-sixth and about two-thirds of the height of the vessel body. In other embodiments, the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-third and about one half of the height of the vessel body. In some embodiments, a retracted impeller assembly remains retracted when the adjustment knob structure is released.

[0016] In some embodiments, the impeller assembly further comprises a magnet. In some embodiments, no magnetic interference occurs with magnetic beads when the impeller assembly is in a maximumly retracted state.

[0017] In some embodiments, a first o-ring is connected to the first end of the rod and the first end of the rod is inside an inner channel of the shaft of the impeller assembly. In some embodiments, the impeller assembly is freely rotatable against the rod.

[0018] According to some aspects of the present disclosure, a bioprocess vessel is provided that comprises a vessel body comprising a top portion and a bottom portion, one or more necked access ports, an impeller assembly comprising a shaft and a plurality of planar blades extending from the shaft, a rod comprising a first end and a second end, and a lever that extends through one of the one or more necked access ports. With this aspect, the impeller assembly shaft is connected to the first end of the rod, the rod extends through a first screw comprising a reversible connection fitting for a second screw attached to an interior surface of the top portion of the vessel body at the center of the interior surface, and an o-ring reversibly connects the second screw to the second end of the rod. Further, the impeller assembly is partially retractable and the bioprocess vessel is configured for activating and expanding T-cells.

[0019] In some embodiments, the shaft comprises at least one hole that the lever can fit within. In some embodiments, rotating the lever in the at least one hole in a first direction disconnects the first screw from the second screw to extend the impeller assembly and rotating the lever in a second direction connects the first screw with the second screw to retract the impeller assembly. In some embodiments, the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-sixth and about two-thirds of the height of the vessel body. In other embodiments, the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-third and about one half of the height of the vessel body.

[0020] In some embodiments, the impeller assembly further comprises a magnet. In some embodiments, no magnetic interference occurs with magnetic beads when the impeller assembly is in a maximumly retracted state.

[0021] In some embodiments, a first o-ring is connected to the first end of the rod and the first end of the rod is inside an inner channel of the shaft of the impeller assembly. In some embodiments, the impeller assembly is freely rotatable against the rod.

[0022] According to some aspects of the present disclosure, a method for activating and expanding T-cells is provided that comprises the steps of: (a) providing a bioprocess vessel of any of the embodiments described above, (b) providing an aqueous solution to the bioprocess vessel that is suitable for T-cell activation and expansion, (c) providing beads to the bioprocess vessel, wherein the beads comprise a T-cell activator, (d) providing T-cells to the bioprocess vessel, and (e) culturing T-cells activated by the beads in the bioprocess vessel. In some embodiments, steps (a) and (b) are performed before step (c), and step (d) is performed after step (c). In some embodiments, steps (a) and (b) are performed before step (d), and step (c) is performed after step (d). In some embodiments, the method further comprises the step of (f) mixing the aqueous solution with the impeller of the impeller assembly.

[0023] In some embodiments, the impeller comprises a magnet in a lower portion of the impeller. In some embodiments, the beads are magnetic beads, and wherein the magnetic beads do not attach magnetically to the impeller magnet during activation and expansion.

[0024] In some embodiments, the T-cells provided to the bioprocess vessel are engineered T-cells. In some embodiments, the engineered T-cells comprise a gene for a chimeric antigen receptor or for a T-cell receptor. In some embodiments, the T-cell activator is an antigen to an $\alpha\beta$ -T-cell receptor on the T-cells. In some embodiments, the antigen comprises anti-CD3 or anti-CD28, or a combination thereof.

[0025] Additional features and advantages will be set forth in the detailed description which follows, and in part will be readily apparent to those skilled in the art from that description or recognized by practicing the embodiments as described herein, including the detailed description which follows, the claims, as well as the appended drawings.

[0026] It is to be understood that both the foregoing general description and the following detailed description are merely exemplary and are intended to provide an overview or framework to understanding the nature and character of the claims. The accompanying drawings are included

to provide a further understanding and are incorporated in and constitute a part of this specification. The drawings illustrate one or more embodiment(s), and together with the description serve to explain principles and operation of the various embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] The following is a description of the figures in the accompanying drawings, given purely by way of non-limiting example. The figures are not necessarily to scale, and certain features and certain views of the figures may be shown exaggerated in scale or in schematic in the interest of clarity and conciseness.

[0028] FIG. 1 is a perspective view of an exemplary bioprocess vessel with an adjustment knob structure, according to embodiments.

[0029] FIG. 2 is a perspective view of an exemplary bioprocess vessel with an adjustment knob structure and an impeller in a non-retracted position (the resting state), according to embodiments.

[0030] FIG. 3 is a perspective view of an exemplary bioprocess vessel with an adjustment knob structure and an impeller in a partially retracted position (the retracted state), according to embodiments.

[0031] FIG. 4 is a bottom view of an exemplary bioprocess vessel with a center nub, planar impeller blades, and with baffles on the sidewalls of the vessel body, according to embodiments.

[0032] FIG. 5 is a perspective view of an exemplary bioprocess vessel with baffles on the side wall of the vessel, and an impeller in an extended position with a magnet and positioning nubs to position the impeller, according to embodiments.

[0033] FIG. 6 is a zoomed-in view of an exemplary intersection between a cross-sectional view of a bottom surface of a bioprocess vessel and the lower portion of an impeller, according to embodiments. The bottom surface includes positioning nubs and a central nub. The impeller includes planar blade, a magnet, a cut-out portion on the bottom of the blades to clear the nubs, and an o-ring coupled to the cut-out portion.

[0034] FIG. 7 is an exploded view of components of a bioprocess vessel, according to embodiments. The bioprocess vessel includes a vessel body, an adjustment knob structure, necked access ports with caps, and an impeller assembly linked to a series of four screws that function to connect an adjustment knob structure at the top of vessel to the blades of the impeller.

[0035] FIG. 8 is an exploded view of components of a bioprocess vessel, according to embodiments. The bioprocess vessel includes a vessel body, an adjustment knob structure, necked access ports with caps, and an impeller assembly linked to a series of three screws that function to connect to a cover, an o-ring, and the adjustment knob structure at the top of the vessel to the blades of the impeller.

[0036] FIG. 9 is an exploded view of components of a bioprocess vessel, according to embodiments. The bioprocess vessel includes a vessel body, an adjustment knob structure, necked access ports with caps, and an impeller assembly linked to the adjustment knob structure through a series of screws, a flexible bag, and a spring inside the flexible bag.

[0037] FIG. 10 is an exploded view of components of a bioprocess vessel, according to embodiments. The bioprocess vessel includes a vessel body, a mechanical lever structure, necked access ports with caps, and an impeller assembly linked to the mechanical lever structure through a series of two screws.

DETAILED DESCRIPTION

[0038] The various aspects and embodiments will now be fully described herein. These aspects and embodiments may, however, be embodied in many different forms and should not be construed as limiting; rather, these embodiments are provided so the disclosure will be thorough and complete, and will fully convey the scope of the present subject matter to those skilled in the art. All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

[0039] Modifications of the disclosure will occur to those skilled in the art and to those who make or use the disclosure. Therefore, it is understood that the embodiments shown in the drawings and described above are merely for illustrative purposes and not intended to limit the

scope of the disclosure, which is defined by the following claims, as interpreted according to the principles of patent law, including the doctrine of equivalents.

A. Definitions

[0040] Unless defined otherwise, all terms and phrases used herein include the meanings that the terms and phrases have attained in the art, unless the contrary is clearly indicated or clearly apparent from the context in which the term or phrase is used. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, particular methods and materials are now described.

[0041] As used herein the terms “the,” “a,” or “an,” mean “at least one,” and should not be limited to “only one” unless explicitly indicated to the contrary. Thus, for example, reference to “a component” includes embodiments having two or more such components unless the context clearly indicates otherwise.

[0042] Unless otherwise stated, the use of individual numerical values is stated as approximations as though the values were preceded by the word “about” or “approximately.” Similarly, the numerical values in the various ranges specified in this application, unless expressly indicated otherwise, are stated as approximations as though the minimum and maximum values within the stated ranges were both preceded by the word “about” or “approximately.” In this manner, variations above and below the stated ranges can be used to achieve substantially the same results as values within the ranges. As used herein, the terms “about” and “approximately” when referring to a numerical value shall have their plain and ordinary meanings to a person of ordinary skill in the art to which the disclosed subject matter is most closely related or the art relevant to the range or element at issue. The amount of broadening from the strict numerical boundary depends upon many factors. For example, some of the factors which may be considered include the criticality of the element and/or the effect a given amount of variation will have on the performance of the claimed subject matter, as well as other considerations known to those of skill in the art. As used herein, the use of differing amounts of significant digits for different numerical values is not meant to limit how the use of the words “about” or “approximately” will serve to broaden a particular numerical value or range. Thus, as a general matter, “about” or “approximately” broaden the numerical value. Also, the disclosure of ranges is intended as a

continuous range including every value between the minimum and maximum values plus the broadening of the range afforded by the use of the term “about” or “approximately.” Consequently, recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, and each separate value is incorporated into the specification as if it were individually recited herein.

[0043] As used herein, the term “and/or,” when used in a list of two or more items, means that any one of the listed items can be employed by itself, or any combination of two or more of the listed items can be employed. For example, if a composition is described as containing components A, B, and/or C, the composition can contain A alone; B alone; C alone; A and B in combination; A and C in combination; B and C in combination; or A, B, and C in combination.

[0044] As used herein, “have,” “having,” “include,” “including,” “comprise,” “comprising” or the like are used in their open ended sense, and generally mean “including, but not limited to.”

[0045] “Optional” or “optionally” means that the subsequently described element, component or circumstance may or may not occur, so that the description includes instances where the element, component, or circumstance occurs and instances where it does not.

[0046] In this document, relational terms, such as first and second, top and bottom, and the like, are used solely to distinguish one entity or action from another entity or action, without necessarily requiring or implying any actual such relationship or order between such entities or actions.

[0047] All scientific and technical terms used herein have meanings commonly used in the art unless otherwise specified. The definitions provided herein are to facilitate understanding of certain terms used frequently herein and are not meant to limit the scope of the present disclosure.

B. Introduction

[0048] Spinner flasks are a low-cost option for culturing cells; however, current spinner flasks lack compatibility with magnetic bead-based T-cell activation techniques. Antibody-conjugated magnetic beads are widely used for activating T-cells in engineered T-cell

immunotherapy production, but spinner flasks have a magnetic impeller that interferes with bead-type T-cell activation techniques. Further, spinner flasks with magnetic impellers impede the use of antibody-conjugated magnetic beads for T-cell activation due to magnetic attraction and repulsion forces between the magnetic beads and the magnetic impeller.

[0049] The present disclosure provides a singular vessel for T-cell activation using all bead types (including magnetic beads), engineering of the T-cells, and expansion of the modified T-cells for patient-derived T-cell immunotherapies. The singular vessel comprises an at least partially retractable impeller that does not interfere with bead-based T-cell activation. The retractable impeller allows bead-based T-cell activation to occur in spinner flasks, including antibody-conjugated/antigen-conjugated magnetic bead based T-cell activation. The retractable impeller allows for T-cell activation to occur in the same vessel as engineering of the T-cells and expansion of the modified T-cells. This closed system vessel minimizes the risks of contamination and reduces the number of handling steps required to create engineered T-cell therapies using spinner flasks.

[0050] Additional features and advantages will be set forth in the detailed description which follows and will be apparent to those skilled in the art from the description or recognized by practicing the embodiments as described in the following description, together with the claims and appended drawings.

C. Bioprocess Vessel

[0051] Referring to FIGS. 1-3, exemplary bioprocess vessels 6 for engineering, activating, and expanding T-cells are shown, according to aspects of the disclosure. Bioprocess vessel 6 comprises a vessel body 10, at least one necked access port (*e.g.*, 16*a*, 16*b*), at least one cap (*e.g.*, 14*a*, 14*b*), an impeller assembly 30, and an adjustment knob structure 18 that translates the impeller assembly 30 along a central axis of the bioprocess vessel 6 in the vertical direction. Vessel body 10 further comprises a top portion 7, which encompasses the end of bioprocess vessel 6 having adjustment knob structure 18, and a bottom portion 8, which encompasses the end of bioprocess vessel 6 opposite of the top portion. In some embodiments, bioprocess vessel 6 has baffles 12 that protrude towards the inside of the bioprocess vessel, as can be seen in FIGS. 1 and 4. The bioprocess vessels typically range in size from volumes of about 125 mL to about 50 liters, but

smaller or larger sizes are also contemplated. In one embodiment, the bioprocess vessel size is about half a liter, about one liter, about two liters, about three liters, about five liters, about 10 liters, about 20 liters, about 25 liters, about 35 liters, or about 50 liters.

[0052] The impeller assembly 30 of the present disclosure includes an impeller blade arrangement 20 and an impeller shaft 26 extending along a vertical axis from adjustment knob structure 18 towards the bottom of bioprocess vessel 6. The shaft 26 may be flexible, rigid, solid, or possess other varying amounts of flexibility such as a series of spiral groove structures in its top end. Extending from shaft 26 is blade arrangement 20, which extends towards the bottom of bioprocess vessel 6. The blade arrangement 20 comprises a plurality of planar blades. In one embodiment, the impeller assembly 30 comprises a shaft 26 and a plurality of planar blades extending from the shaft 26. In the embodiments shown in FIGS. 2-3, blade arrangement 20 has four planar blades, each disposed 90 degrees relative to each other. Of the four planar blades, there are two major blades 50 and two minor blades 54. The major blades 50 are disposed 180 degrees relative to one another and likewise, the two minor blades 54 are disposed 180 degrees relative to each another. The minor blades are smaller than the major blades. The blade arrangement 20 of major and minor blades around shaft 26 creates an alternating effect of a minor-major blade orientation. However, as understood by those with ordinary skill in the art, other blade arrangements can be employed in this disclosure, including those that employ fewer or more than four blades, and those with different shapes and sizes. For example, a blade arrangement may comprise two blades of the same size or of unequal sizes. In another example, a blade arrangement may comprise three blades, each with a different shape.

[0053] With reference to FIG. 4, in some embodiments, at the bottom of shaft 26 and beneath the major and minor blades 50, 54 of the impeller assembly is an impeller o-ring 40. The impeller o-ring 40 is coupled to a bottom cut-out portion of the planar blades. In some embodiments, the bottom edge of the impeller o-ring is even with a bottom edge of the planar blades 50, 54. In other embodiments, the bottom edge of the impeller o-ring is below the bottom edge of the planar blades 50, 54. A plurality of positioning nubs 38 are coupled to the bottom interior surface spaced from or along an inside edge 40a of the impeller o-ring 40. In some embodiments, the group of positioning nubs can have two, three, four, five, six, or more positioning nubs 38 spaced apart from each other along the inside edge 40a of the impeller o-ring

40. In some embodiments, the plurality of positioning nubs 38 may be spaced an equal distance from one another along the inside edge 40a of the impeller o-ring 40. In one embodiment, the plurality of positioning nubs is three positioning nubs 38, each nub spaced an equal distance from one another. The plurality of positioning nubs can have each nub spaced an equal distance from one another along the inside edge 40a of the impeller o-ring 40 or may have each nub spaced at unequal distances from one another. FIG. 4 further depicts that baffles 12 of the bioprocess vessel protrude inward from the vessel body 10.

[0054] In some embodiments, the plurality of positioning nubs 38 may comprise individual nubs that have a cylindrical (*e.g.*, character reference 96 in FIG. 5), block, square, conical (*e.g.*, character reference 94 in FIG. 6), rectangular, or pyramidal shape. The plurality of positioning nubs 38 can also have other shapes or combinations of shapes. In certain aspects, the shape of the positioning nubs 38 can be configured to make constant, periodic, or frequent contact with the inside edge 40a of the impeller o-ring 40 to prevent perpendicular or lateral movement of the impeller assembly.

[0055] In some embodiments, there is a center nub 42 on the bottom, interior surface of the bioprocess vessel. The center nub 42 is a raised feature, as shown in FIGS. 5-6, and can comprise various shapes. For example, the center nub 42 may include cylindrical (see FIG. 5), block, square, conical (see FIG. 6), rectangular, pyramidal, or any other shaped nub. In some embodiments, the center nub 42 can have rounded or beveled edges. The center nub 42 serves at least two purposes: (1) to prevent cells and/or beads from pooling or grouping together below the impeller assembly; and (2) to minimize the perpendicular or lateral movement of the impeller assembly during shipment and spinning to avoid impeller breakage. Although the raised center nub 42 can help minimize the perpendicular or lateral movement of the impeller assembly, the positioning nubs 38 may also help minimize the perpendicular or lateral movement of the impeller assembly.

[0056] The impeller assembly of the present disclosure may sometimes be positioned at a resting state (that is, a non-retracted position) and the bioprocess vessel may be used for T-cell engineering and expansion during the resting state. With the resting state, in some embodiments, the distance between the top surface of the center nub 42 and the bottom of the impeller assembly

can be from about 0.001 inches to about 0.1 inches. In other embodiments, the distance between the top of the center nub 42 and the bottom of the impeller assembly can be from about 0.005 inches to about 0.05 inches. In still other embodiments, the distance between the top of the center nub 42 and the bottom of the impeller assembly can be from about 0.01 inches to about 0.03 inches. For example, the distance between the top of the center nub 42 and the bottom of the impeller assembly can be about 0.0150 inches, about 0.0185 inches, about 0.0200 inches, about 0.0250 inches, or all distance values between these recited values and ranges disclosed herein. In some embodiments, the tolerance for the plurality of positioning nubs 38, the center nub 42, the impeller assembly, and other dimensional features of the bioprocess vessel is from ± 0.001 inches to ± 0.100 inches. In one specific embodiment, the tolerance for the plurality of positioning nubs 38, the center nub 42, the impeller assembly, and other dimensional features of the bioprocess vessel is about ± 0.005 inches.

[0057] The impeller assembly of the present disclosure may sometimes be positioned at a retracted state and the bioprocess vessel may be used for bead-based T-cell activation during the retracted state. In the retracted state, the impeller assembly is retracted away from the bottom interior surface of the bioprocess vessel, so the impeller assembly will not interfere with the bead-based T-cell activation process. The distance between the top surface of the center nub 42 and the bottom of the impeller assembly is dependent on the dimension of the bioprocess vessel as well as the medium volume during the T-cell activation step. In the retracted state, in some embodiments, the impeller assembly is retracted away from the bottom interior surface of the bioprocess vessel, where it is in its fully extended position, up to a distance between about 1/6 (one-sixth) and about 2/3 (two-thirds) of the height of the vessel body 10. The height of the vessel body includes the top and bottom portions of the vessel body but excludes any adjustment knob structure for translating the impeller assembly vertically and any necked access ports on the top portion of the vessel body. In some embodiments, the impeller assembly is retracted away from the bottom interior surface of the bioprocess vessel up to a distance between about 1/3 (one-third) and about 1/2 (half) of the height of the vessel body 10.

[0058] With either the resting state or retracted state, the distance between the base outer diameter (diameter of the center nub where connected to bottom interior surface) of the center nub 42 and the inner diameter of the impeller o-ring 40 may be from about 0.01 inches to about 1.0

inches. In other embodiments, the distance between the base outer diameter of the center nub 42 and the inner diameter of the impeller o-ring 40 may be from about 0.1 inches to about 0.5 inches. In still other embodiments, the distance between the base outer diameter of the center nub 42 and the inner diameter of the impeller o-ring 40 may be from about 0.2 inches to about 0.3 inches. For example, the distance between the base outer diameter of the center nub 42 and the inner diameter of the impeller o-ring 40 may be about 0.15 inches, about 0.20 inches, about 0.025 inches, about 0.030 inches, about 0.035 inches, or any value or range between 0.01 inches and 1.0 inches.

[0059] The bioprocess vessels for culturing cells disclosed herein have the ability to offer a readily available, affordable, disposable, pre-sterilized, fully integrated cell culture vessel which provides gentle stirring to minimize hydrodynamic shearing and keep cells suspended in the bioprocess vessel. A center nub and/or positioning nubs on the bottom interior surface of the bioprocess vessel can prevent pooling of beads and/or cells below the impeller assembly and also can minimize the perpendicular movement of the impeller assembly during shipping and spinning to prevent damage to the beads, cells, and/or impeller assembly.

[0060] Regarding impeller size, the impeller assembly 30 may be sized such that the blades 50, 54 extend nearly the full diameter of bioprocess vessel 6. In one embodiment, the impeller blades 50, 54 extend approximately 50-95% of the bioprocess vessel's radius, as measured from the midpoint of a cross sectional area between the vertical sidewalls of the inside of the vessel body. In another embodiment, at least one blade 50, 54 extends 75-95% of the bioprocess vessel's radius, as measured from the midpoint of a cross sectional area between the vertical sidewalls of the inside of the vessel body. However, it should be understood that the impeller blades may extend any distance, including less than 50% of the bioprocess vessel's radius, or even greater than 95% but less than 100% of the bioprocess vessel's radius, as measured from the midpoint of a cross sectional area between the vertical sidewalls of the inside of the vessel body.

[0061] The bottom portion of the impeller assembly (the end nearest the bottom of the bioprocess vessel) has a cut-out portion that is shaped within the intersection of the bottom edges of the respective impeller blades. The bottom cut-out portion substantially follows the contour of the center nub. For example, if the center nub is cylindrical in shape, the bottom cut-out portion substantially follows the cylindrical shape of the center nub, as shown in FIG. 5. As another

example, if the center nub is conical in shape, the bottom cut-out portion substantially follows the conical shape of the center nub, as shown in FIG. 6.

[0062] The impeller o-ring coupled to the bottom cut-out portion of the impeller assembly is not intended to contact the central nub when the bioprocess vessel is positioned in an upright position. In some embodiments, there may be no contact points between the impeller assembly and the bioprocess vessel below the impeller shaft receptacle. This is advantageous in that it helps reduce the possibility of cell damage if cells or beads are caught between the bottom edges of the planar impeller blades and the center nub, or due to shear stresses. The central nub on the bottom interior surface 70 of the bioprocess vessel also eliminates any potential dead spots (spots where turbulence created by the spinning impeller is at a minimum) directly below the central axis of the impeller assembly. The bottom cut-out portion within the intersection point of the impeller blades 50, 54 allows the blade edges 66 to come into close proximity with the bottom interior surface 70, as shown in the blown-up view in FIG. 6. In one embodiment, the distance between the impeller blades 50, 54 and the bottom interior surface 70 of the bioprocess vessel is between about 0.05 inches and about 0.5 inches. Since the bioprocess vessel is intended to be shipped as an integral unit, the impeller o-ring in combination with the center nub also serves to contain the impeller assembly during shipping in such a way that the impeller assembly cannot disengage from the bottom of the bioprocess vessel or damage the sidewall of the vessel body through contact caused by jostling of the bioprocess vessel.

[0063] In some embodiments, the impeller has a magnet in the bottom portion of the impeller. As shown in FIG. 4 and FIG. 6, a magnet receptacle 24 for receiving a magnetic stir bar (magnet) 22 is molded into each lower portion of the two major blades 50. A hole in the minor blades 54 and shaft 26 area completes the magnet receptacle 24. A cylindrical plug or magnetic stir bar 22 is mounted in the magnet receptacle 24 along the lower edge of the two major blades 50 and orthogonal to the minor blades 54. Alternatively, the magnet 22 itself is molded into the impeller assembly. To accomplish this, the magnet 22 is inserted into a mold and the impeller assembly is over-molded around the magnet 22 itself. Having the magnet 22 integrally molded within the impeller assembly provides the advantage that during assembly and shipping, the magnet 22 cannot detach from the impeller assembly and damage the bioprocess vessel.

[0064] With reference to FIG. 7, in some embodiments a rod 100 has a first end with an o-ring (not shown) and a second end with a series of spiral grooves 101. The rod 100 is placed inside an inner channel of the center shaft 26 so that the blade arrangement 20 is attached to the o-ring containing end of the rod 100 and is freely rotatable against the rod 100, while the spiral groove containing end of the rod extends from the top end of the center shaft 26. For the impeller assembly 30, the rod 100 passes through a first screw structure 110 having a series of spiral grooves outside of its top portion, the port 16c of the top part 74 of the top portion 7 of the vessel body 10, a second screw structure 111 having a series of spiral grooves inside its channel, a third screw structure 112, an adjustment knob structure 18 having a series of spiral grooves inside its channel, and finally a fourth screw structure 113. The first screw structure 110 fits with the inner spiral grooves of the second screw structure 111 and can fasten together with the top part 74 of the top portion 7 of the vessel body 10. The third screw structure 112 attaches the adjustment knob structure 18 to the top part 74 of the top portion 7 of the vessel body 10 to establish a closed system. The fourth screw structure 113 matches the spiral grooves of the top end of the rod 100 and holds the entire impeller assembly in place so that the entire impeller assembly can be adjusted to a predefined height (i.e., the resting state or the retracted state) from the bottom of the bioprocess vessel 6 via tightening or loosening the fourth screw structure 113. Tightening retracts the impeller assembly while loosening lowers it. Additional o-rings can be placed among different layers or structures to form tight sealing in order to achieve a tighter closed system. In some embodiments, the third screw structure 112 is absent, and the adjustment knob structure 18 is directly in contact with the second screw structure 111 to form a tight seal. In some embodiments, the adjustment knob structure 18 and the fourth screw structure 113 are enclosed within a soft bag-like structure so the entire vessel remains to be a closed system during the operation of T-cell activation and expansion process where the impeller assembly need to be retracted first and then not retracted.

[0065] With reference to FIG. 8, in some embodiments a rod 100 has a first end with an o-ring (not shown) and is placed inside an inner channel of the center shaft 26 so that the blade arrangement 20 is attached to the first end of the rod 100 and is freely rotatable against the rod 100. A second end of the rod 100 extends from the center shaft 26, passes through a first screw structure 110 having a series of spiral grooves outside of its top portion, the port 16c of the top part 74 of the top portion 7 of the vessel body 10, a second screw structure 111 having a series of spiral grooves inside its channel, a third screw structure 112 having a series of spiral grooves

outside the structure, an adjustment knob structure 18 having a series of spiral grooves inside its channel, an o-ring 121 attached the second end of the rod 100, and finally a cover 18a to form a closed system. The o-ring 121 in the second end of the rod 100 allows the entire impeller assembly to be attached to the adjustment knob structure 18. The first screw structure 110 fits with the inner spiral grooves of the second screw structure 111 and can fasten together with the top part 74 of the top portion 7 of the vessel body 10. The outer spiral grooves of the third screw structure 112 matches with the inner spiral grooves of the second screw structure 111, as well as the inner spiral grooves of the adjustment knob structure 18. In this way, the entire impeller assembly can be adjusted from a position near the bottom of the bioprocess vessel 6 to a predefined height (i.e., the resting state or a retracted state) via tightening or loosening the adjustment knob structure 18 against the third screw structure 112. Tightening retracts the impeller assembly while loosening lowers the impeller assembly. Additional o-rings can be placed among different layers or structures to form better sealing to achieve an even tighter closed system. For the o-rings described in any of the embodiments herein, the o-rings may be made from PTFE (polytetrafluoroethylene), nylon or other similar low-friction material.

[0066] With reference to FIG. 9, in some embodiments a rod 100 has a first end with an o-ring (not shown) and is placed inside an inner channel of the center shaft 26 so that the blade arrangement 20 is attached to the first end of the rod 100 and is freely rotatable against the rod 100. A second end of the rod 100 extends from the center shaft 26, passes through a first screw structure 110 having a series of spiral grooves outside of its top portion, the port 16c of the top part 74 of the top portion 7 of the vessel body 10, a bottom of a flexible bag-like structure 120, a second screw structure 111 having a series of spiral grooves inside its channel, a third screw structure 112 having a series of spiral grooves outside the structure, an o-ring attached the second end of the rod 100, a spring 115, a top of the flexible bag-like structure 120, and an adjustment knob structure 18 having a series of spiral grooves inside its channel. The first screw structure 110 fits with the inner spiral grooves of the second screw structure 111 and can fasten together with the top part 74 of the top portion 7 of the vessel body 10. The inner spiral grooves of the adjustment knob structure 18 matches with the outer spiral grooves of the third screw structure 112 so that the entire impeller assembly is attached to the adjustment knob structure 18. When the third screw structure 112 connects the adjustment knob structure with the top part 74 of the top portion 7 of the vessel body 10, the spring 115 is compressed so the entire impeller assembly is at the resting

state (i.e., the closest to the bottom of the bioprocess vessel 6). When the third screw structure 112 is detached from the second screw structure 111 (that is, detached from the top part 74 of the top portion 7 of the bioprocess vessel 6), the spring is relaxed, so the entire impeller assembly is a retracted state (i.e., the impeller assembly is brought away from the bottom of the bioprocess vessel 6). The flexible bag-like structure 120 is to ensure the whole bioprocess vessel is a closed system regardless of the impeller assembly state. Additionally, o-rings can be placed among different layers or structures to form better sealing to achieve a tighter closed system.

[0067] With reference to FIG. 10, in some embodiments a rod 100 has a first end with an o-ring and is placed inside an inner channel of the center shaft 26 so that the blade arrangement 10 is attached to the first end of the rod 100 and is freely rotatable against the rod 100. A second end of the rod 100 extends from the center shaft 26, passes through a first screw structure 110 having a series of spiral grooves outside of its top portion, and an o-ring 118 that is attached to the second end of the rod 100. The o-ring has a dimension smaller than the diameter of the top end of the first screw structure 110 and serves as a mechanical means to hold the impeller assembly against the first screw structure 110, while it does not interfere with the insertion of the first screw structure 110 into the second screw structure 111. The first screw structure 110 is then inserted into the second screw structure 111 by rotating to fasten together, where the second screw structure 111 is fixed underneath the top portion 7 of the vessel body 10 in its center location so that the entire impeller assembly is attached to the top portion of the vessel. A lever 120 is inserted in a necked access port 16a via its cap 14a and hangs freely, when the impeller assembly is at the resting state (i.e., close to the bottom of the bioprocess vessel 6). When the second end of the lever 120 is inserted into the hole in the side of the top end of the center shaft 26 and turned, the impeller assembly can be retracted away from the bottom of the bioprocess vessel 6. O-rings can be placed among between the first and second screw structures to form better sealing to achieve a tighter closed system.

[0068] The greatest height that can be achieved for the impeller assembly above the bottom of the interior surface of the bioprocess vessel is dependent on the location on the impeller shaft where the impeller blades begin extending from the impeller shaft, as well as the bioprocess vessel design. The blades themselves are too large to fit through the threads on the knob receptacle, and so the maximum height above the bottom of the interior surface will be at or slightly less than

where the blades extend from the shaft. When the bioprocess vessel is assembled according to FIG. 7, the greatest retracted height is also dependent on the length of the spiral grooves 101 of the second end of the rod 100. When the bioprocess vessel is assembled according to FIG. 8, the maximal retracted height is also dependent on the length of the third screw structure 112. When the bioprocess vessel is assembled according to FIG. 9, the maximal retracted height is also dependent on the relaxed length of the spring structure 115. In contrast, when the bioprocess vessel is assembled according to FIG. 10, the maximal retracted height is also dependent on the distance that the lever 120 can raise the impeller assembly up. Further, when the impeller assembly is retracted out of the medium, a static condition (i.e., the blades are not spinning in fluid) is achieved, and magnetic forces between the magnetic beads and any magnet of the impeller assembly will be reduced or eliminated. This allows for magnetic beads carrying T-cell activators to be used with the bioprocess vessel. In some embodiments, when the impeller assembly is maximally retracted, no magnetic interference between the magnetic beads and the impeller magnet occurs. As used herein, “magnetic interference” means when magnetic beads magnetically attach to an impeller magnet.

[0069] As shown in FIGS. 1-3, one or more necked access ports 16 extend outward from the top portion 7 of the bioprocess vessel 6. In some embodiments, one or more of the caps 14 may removably attach to necked access ports 16. In one embodiment, at least one internally threaded cap is removably attached to an exteriorly threaded necked access port. In some embodiments, as shown for example in FIG. 7-10, at least one cap 14 has a vent 75 that allows the necessary gaseous communication with the external environment. In some embodiments, the cap has internal threading and is removably attached to a necked access port that has external threading, and the cap further comprises a vent. In another embodiment, accessories such as tubes may be employed and connected to the one or more necked access ports 16 to allow aseptic dispensing. In another embodiment, as shown for example in FIG. 7, the cap 14 comprises a hydrophobic membrane insert 80 made from material that will allow gas transport into the bioprocess vessel interior but prevent liquid from escaping the bioprocess vessel 6 and other contaminants from entering the bioprocess vessel 6. Examples of such membrane material include polytetrafluoroethylene and polyvinylidene fluoride (PVDF).

[0070] In some embodiments, like those shown for example in FIGS. 1-3, and 7-10, the one or more necked access ports 16 extend at an angle from horizontal to allow instruments such as pipettes to pass by impeller assembly and reach adjacent regions of stirring vessels having preselected depths. Nevertheless, the dimensions of the one or more access ports 16 and the angles in which the access ports 16 extend from the vessel body 10 may be selected to optimize instrument accessibility to regions within various bioprocess vessels 6. Further, in some embodiments, the one or more access ports is two access ports 16 as disclosed for example in FIGS. 1-3 and 7-10, but it will be appreciated that any number of ports 16 are possible.

[0071] In some embodiments, like those shown in the figures, a plurality of baffles 12 extends along the interior wall of the vessel body 10 in a vertical direction that is parallel to the central axis of the bioprocess vessel 6. In some embodiments, like those shown in the figures, baffle 12 has roughly the cross sectional shape of a half-cylinder or an isosceles triangle. Each baffle 12 originates from the bioprocess vessel bottom and extends vertically upward terminating in an elliptical shape (see character reference 56 in FIG. 5). While the baffles 12 illustrated herein are shown to terminate in an elliptical shape, embodiments of the present disclosure are not so limited, and any shape may be employed. It is believed that having the plurality of baffles 12 extend completely through the liquid region (i.e. from the bottom interior surface 70 of vessel body 10 to a point above the liquid surface) enhances turbulence throughout the entire liquid domain. The plurality of baffles 12 project into the bioprocess vessel cavity, in combination with the impeller assembly 30, create and enable turbulence with the bioprocess vessel interior. The plurality of baffles 12 are preferably formed integrally with the wall of the container. In some embodiments, the plurality of baffles 12 is three baffles 12, each baffle is spaced an equal distance from each of the other baffles around a perimeter defined by a cylindrical sidewall of vessel body 10. In other aspects, the plurality of baffles is disposed symmetrically along an interior cylindrical sidewall of vessel body 10, but the number and density of baffles 12 may vary based on the bioprocess vessel size. In embodiments, each baffle is integral with the sidewall of the vessel body originating at the bottom interior surface and extending vertically to a predetermined distance up the sidewall.

[0072] In some embodiments, the bioprocess vessel of the present disclosure is made from an injection molded polymer, for example polystyrene, polycarbonate or any other appropriate

polymer as identified by one of skill in the art. In one embodiment, the polymer is optically transparent and non-cytotoxic. Since the materials are made from lightweight polymers and the bioprocess vessel is pre-sterilized during manufacture, the bioprocess vessel itself is disposable and there is no need for the end user to sterilize components of the system prior to use. In some embodiments, the main body (that is the bottom portion 8) of the vessel body 10 of the present disclosure is made from glass, and the top portion 7 of the vessel body 10 is made of plastics.

[0073] In describing the manufacturing and assembly process, the impeller assembly 30, the top portion 7, and the bottom portion 8 of the vessel body 10 may be molded separately and treated as discussed. Thereafter, in embodiments where the magnet 22 is not over-molded, the magnet 22 is placed in the magnet receptacle 24. In embodiments where the magnet 22 itself is over-molded and therefore integral with the blade arrangement 20, the magnet is added at the molding stage. The blade assembly 20 is then attached to rod 100, which is connected to the top portion 7 of the vessel body 10 through adjustment knob structure 18 according to embodiments shown in FIG. 7-9. The o-ring 40 is slipped over the end of impeller shaft 26 and contacted with a receiving groove. In some embodiments, the top portion 7 and the bottom portion 8 of the vessel body 10 are then permanently affixed to one another by, for example, ultrasonic welding along a weld line thereby creating a completely and permanently integral unit. In other embodiments, the parts are laser welded or attached by means of adhesives. In embodiments having one or more necked access ports 16 and caps 14, the caps 14 are put into place and the unit is effectively sealed for shipment. The integral unit may then be sterilized. In some embodiments the top portion 7 is screwed to the bottom portion 8 of the vessel to form tight seal. As most cell culture procedures are carried out under aseptic conditions by practicing the so-called sterile technique, the pre-sterilization of the bioprocess vessel 6 provides the culture chamber to be maintained in a sterile, closed environment. It is advantageous to have the cell culture process carried out in a system where the culture chamber is functionally closed to the external environment, with the sterile integrity maintained from the time the vessel is manufactured until it has been disposed of. One method of pre-sterilizing includes gamma irradiation. Other sterilization methods known to those skilled in the art including ethylene oxide or electron beam irradiation treatment could also be used.

[0074] The bioprocess vessels of the present disclosure allow for the engineering, activation (and expansion) of engineered T-cells. Activation is a step that currently available spinner flasks are unable to successfully perform due to interference between the impeller blades and beads that carry the T-cell activators. The bioprocess vessel of the present disclosure solves this problem by providing a spinner flask that can successfully perform T-cell activation using beads. The present disclosure provides a retractable impeller assembly that does not interfere with the beads during activation when the impeller assembly is retracted away from the bottom surface of the vessel. Methods of activating and expanding engineered T-cells with the bioprocess vessels of the present disclosure will now be described.

[0075] A method of activating and expanding T-cells generally comprises the steps of (1) providing one of the bioprocess vessels of the present disclosure, (2) providing an aqueous solution to the bioprocess vessel that is suitable for T-cell activation and expansion, (3) providing T-cell activators to the bioprocess vessel, (4) providing T-cells to the bioprocess vessel, (5) culturing T-cells activated by the beads in the bioprocess vessel when the impeller assembly is retracted out of the solution, and (6) culturing the activated T-cells to expand the cells under the spinning culture condition after the impeller assembly is re-set to the resting state and spin. Any of the embodiments for the bioprocess vessel described above will work for activating and expanding T-cells, including engineered T-cells.

[0076] The aqueous solutions that can be used for T-cell activation and expansion are well-known to those skilled in the field of T-cells. Non-limiting examples include various cell culture media, such as Roswell Park Memorial Institute (“RPMI”) 1640 medium (Gibco®), HyClone® RPMI 1640 medium (Cytiva®), Iscov’s Modified Dulbecc’s Medium (“IMDM”), OpTmizer® CTS® T Cell Expansion Serum-Free Medium (Gibco), CTS® AIM-V® medium (Gibco®), X-VIVO® 15 (Lonza®), and StemSpan® Serum-Free Expansion Medium or StemSpan® SFEM II medium (StemSpan® Technologies Canada, Inc.), and other like-media, including serum containing and serum-free variations, and other supplemented variations (e.g., with added L-glutamine, glucose, etc.).

[0077] A T-cell activator is a reagent that stimulates T-cells to initiate targeted intracellular signals that increase a desired result (for example, overexpression of certain receptors on the

outside of an engineered T-cell). Common examples include but are not limited to antigens for $\alpha\beta$ -T-cell receptors (e.g., anti-CD3 monoclonal antibodies, anti-CD28 monoclonal antibodies, anti-CD19 monoclonal antibodies, and other like antigens), phytohemagglutinin (PHA) mitogen, certain cytokines such as IL-2 (interleukin-2), and combinations thereof. Any T-cell activator known to those skilled in the field of T-cells can be provided to the bioprocess vessel of the present disclosure. To create T-cell signaling that extends long enough to lead to productive responses, in some embodiments, the T-cell activators may be surface-bound to mimic cellular interactions. In one embodiment, the surface-bound T-cell activators may be bead-based, meaning the T-cell activators are coated or attached to the exterior surface of beads that can be provided to the bioprocess vessel. In one specific embodiment, the beads are magnetic beads. In another specific embodiment, the beads are polystyrene beads. In another specific embodiment, the beads are glass beads. Any known method of attaching the activator to the surface of the beads may be used, as understood by those skilled in the art of T-cells.

[0078] The T-cells provided to the vessel may be any type of T-cell. In some embodiments, the T-cell is an engineered T-cell. Engineered T-cells include T-cells that have been transduced with a viral vector that carries desired genes, such as CAR or TCR. However, any T-cell that has been genetically altered is an engineered T-cell. In some embodiments, the T-cells are non-engineered T-cells. Non-limiting examples of such T-cells include patient derived CD3⁺ T-cells, or other patient derived T-cells.

[0079] To operate the bioprocess vessel of the present disclosure for T-cell activation and expansion, the aqueous solution, T-cells, and T-cell activators are all added to the bioprocess vessel. The first step of the method is to provide the bioprocess vessel of the present disclosure. The next step of the method is providing to the bioreactor vessel, in any order, the aqueous solution, the T-cells, and the T-cell activator. In one embodiment, the aqueous solution is provided before the T-cells, and the T-cells are provided before the T-cell activator. In another embodiment, the aqueous solution is provided before the T-cell activator, and the T-cell activator is provided before the T-cells. In another embodiment, the T-cell activator is provided before the T-cells, and the T-cells are provided before the aqueous solution. In another embodiment, the T-cell activator is provided before the aqueous solution, and the aqueous solution is provided before the T-cells. In yet another embodiment, the T-cells are provided before the T-cell activator, and the T-cell

activator is provided before the aqueous solution. In another embodiment, the T-cells are provided before the aqueous solution, and the aqueous solution is provided before the T-cell activator. Any other ordering of aqueous solution, T-cells, and T-cell activators can be performed.

[0080] However, in embodiments where beads are used as the T-cell activator, the impeller assembly of the bioprocess vessel should be retracted before the beads are provided to prevent damage to the beads or any coatings that may be on the beads. In embodiments using magnetic beads, retracting of the impeller before adding the beads further has the advantage of reducing or eliminating magnetic forces from interfering with the beads so they do not attract or stick to any magnet in the impeller assembly.

[0081] The T-cells, T-cell activators, and aqueous solution (such as a culture medium) are delivered through the one or more access ports 16 of the container. The aqueous liquid is added until it reaches a desired fluid level in the bioprocess vessel (i.e., bioprocess vessel 6 in the figures). Preferably, the fluid level is below the top edge of the blades 50, 54 and the top of the baffles 12 but above the lower portion (where the blades fan out the furthest from the center) of the major blades 50.

[0082] In embodiments where the impeller assembly comprises a magnet, once the aqueous liquid is in the bioprocess vessel 6, a magnetic stirring device (not shown) may be used with the bioprocess vessel 6 and the stirring device causes the magnetic stir bar 22 to spin within the bioprocess vessel 6. As a result, the impeller assembly 30 including the shaft 26 and the blades 50, 54 is also rotated within the bioprocess vessel 6. The rotation of the blade arrangement 20 causes the fluid to stir within the container. Alternatively, the blade assembly 20 (with or without a magnet) may be rotated by a motorized mechanism engaging the top of the shaft 26. The shape of the blades 50, 54 and the interaction with the baffles 12 causes the liquid to circulate from a position near the top of the fluid level to a position near the bottom of the fluid level. The center nub 42 prevents material from accumulating at the center of the bottom interior surface 70 of the bioprocess vessel 6. Since the upper portion 52 of the major blades 50 extends above the fluid level, the surface area of the liquid in the container is effectively increased and continually agitated, resulting in aeration of the liquid. This fluid stirring similarly occurs with any other blade configuration, including those having only one type of blade or having blades of a different shape.

[0083] The impeller assembly of the bioprocess vessel is used to stir T-cells after activation. Preferably, the mixing is effective such that the liquid cycles from the bottom of the apparatus to the surface, and back again. Typically, the cells are maintained at about 27-37° C and mixed at 5 to 300 rpm. However, as understood by those skilled in the art, these conditions can be varied depending on the particular T-cells, T-cell activators, or application. In embodiments where magnetic beads are used as T-cell activators and the impeller assembly has a magnet, the maximum speed used for the impeller assembly may be lower than when a non-magnetic impeller is used or when non-magnetic beads are used. This is to prevent the magnetic beads from rising to a height where attractive magnetic forces would cause the beads to attract magnetically and attach to the retracted impeller. In one specific embodiment, the aqueous solution is mixed at less than about 275 rpm, about 250 rpm, about 225 rpm, about 200 rpm, about 175 rpm, 150 rpm, 125 rpm, 100 rpm, 75 rpm, 50 rpm or 25 rpm, and the magnetic beads do not attach magnetically to the retracted impeller.

[0084] The activation and expansion process may occur over an extended time-period. In one embodiment, the activation and expansion process may occur between 2 hours and 3 months. In another embodiment, the activation and expansion process may occur between 0.5 days and 10 days. In some embodiments, used aqueous solution may be replaced with fresh aqueous solution through the necked access ports on the bioprocess vessel. This is advantageous with activation and expansion processes that typically take several days or weeks as it allows optimal conditions to be maintained throughout the process.

[0085] T-cells or cellular materials may be harvested through the necked access ports by means of pipette, pouring, or pumping. The impeller assembly may be retracted or extended or somewhere in between when the T-cells are harvested.

[0086] While the present disclosure includes a limited number of embodiments, those skilled in the art, having benefit of this disclosure, will appreciate that other embodiments can be devised which do not depart from the scope of the present disclosure.

CLAIMS

What Is Claimed Is:

1. A bioprocess vessel, comprising:

a vessel body comprising a top portion and a bottom portion;

one or more necked access ports;

an impeller assembly comprising a shaft and a plurality of planar blades extending from the shaft;

a rod comprising a first end and a second end; and

an adjustment knob structure;

wherein the shaft of the impeller assembly is connected to the first end of the rod, the rod extends through the adjustment knob structure, and the second end of the rod is connected to a screw external to the adjustment knob structure;

wherein the impeller assembly is partially retractable; and

wherein the bioprocess vessel is configured for activating and expanding T-cells.
2. The bioprocess vessel of claim 1, wherein the second end of the rod comprises a series of spiral grooves that connects to internal spiral grooves on the screw.
3. The bioprocess vessel of claim 1, wherein the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-sixth and about two-thirds of the height of the vessel body.
4. The bioprocess vessel of claim 3, wherein the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-third and about one half of the height of the vessel body.
5. The bioprocess vessel of claim 1, wherein the impeller assembly comprises a magnet.

6. The bioprocess vessel of claim 2, wherein a retracted impeller assembly remains retracted when the screw is released.
7. The bioprocess vessel of claim 5, wherein no magnetic interference occurs with magnetic beads when the impeller assembly is in a maximumly retracted state.
8. The bioprocess vessel of claim 1, wherein a first o-ring is connected to the first end of the rod and the first end of the rod is inside an inner channel of the shaft of the impeller assembly.
9. The bioprocess vessel of claim 8, wherein the impeller assembly is freely rotatable against the rod.
10. A bioprocess vessel, comprising:
 - a vessel body comprising a top portion and a bottom portion;
 - one or more necked access ports;
 - an impeller assembly comprising a shaft and a plurality of planar blades extending from the shaft;
 - a rod comprising a first end and a second end; and
 - an adjustment knob structure;
 - wherein the impeller assembly shaft is connected to the first end of the rod, the rod extends through the adjustment knob structure, and the second end of the rod is connected to a first o-ring that is external to the adjustment knob structure;
 - wherein the impeller assembly is partially retractable; and
 - wherein the bioprocess vessel is configured for activating and expanding T-cells.
11. The bioprocess vessel of claim 10, wherein the adjustment knob structure comprises a series of internal spiral grooves on its inner surface that connects to a screw comprising a series of spiral grooves on its external surface, and wherein the rod extends through the screw.

12. The bioprocess vessel of claim 10, wherein tightening the adjustment knob structure on the screw extends the impeller assembly and loosening the adjustment knob structure on the screw retracts the impeller assembly.
13. The bioprocess vessel of claim 10, wherein the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-sixth and about two-thirds of the height of the vessel body.
14. The bioprocess vessel of claim 13, wherein the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-third and about one half of the height of the vessel body.
15. The bioprocess vessel of claim 10, wherein the impeller assembly comprises a magnet.
16. The bioprocess vessel of claim 10, wherein a retracted impeller assembly remains retracted when the adjustment knob structure is released.
17. The bioprocess vessel of claim 15, wherein no magnetic interference occurs with magnetic beads when the impeller assembly is in a maximumly retracted state.
18. The bioprocess vessel of claim 10, wherein a first o-ring is connected to the first end of the rod and the first end of the rod is inside an inner channel of the shaft of the impeller assembly.
19. The bioprocess vessel of claim 18, wherein the impeller assembly is freely rotatable against the rod.
20. A bioprocess vessel, comprising:
 - a vessel body comprising a top portion and a bottom portion;
 - one or more necked access ports;
 - an impeller assembly comprising a shaft and a plurality of planar blades extending from the shaft;
 - a rod comprising a first end and a second end; and

an adjustment knob structure;

wherein the shaft of the impeller assembly is connected to the first end of the rod, the second end of the rod extends through the top portion of the vessel body and through a first screw connected to an external side of the top portion of the vessel body, and the second end of the rod connects to the interior surface of a second screw comprising a series of spiral grooves on its exterior surface;

wherein the second screw further comprises an exterior surface that reversibly connects to an interior surface of the adjustment knob structure, and wherein the second screw is connected to a spring that is also connected to the adjustment knob structure;

wherein the impeller assembly is partially retractable; and

wherein the bioprocess vessel is configured for activating and expanding T-cells.

21. The bioprocess vessel of claim 20, wherein a flexible bag-like structure is connected the top portion of the bioprocess vessel by the first screw and to the interior surface of the adjustable knob structure, and wherein the rod and the spring and the second screw are inside the flexible bag-like structure.

22. The bioprocess vessel of claim 20, wherein the impeller assembly is retracted by connecting the second screw to the first screw and the impeller assembly is extended by disconnecting the second screw from the first screw.

23. The bioprocess vessel of claim 20, wherein the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-sixth and about two-thirds of the height of the vessel body.

24. The bioprocess vessel of claim 23, wherein the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-third and about one half of the height of the vessel body.

25. The bioprocess vessel of claim 20, wherein the impeller assembly further comprises a magnet.

26. The bioprocess vessel of claim 20, wherein a retracted impeller assembly remains retracted when the adjustment knob structure is released.
27. The bioprocess vessel of claim 25, wherein no magnetic interference occurs with magnetic beads when the impeller assembly is in a maximumly retracted state.
28. The bioprocess vessel of claim 20, wherein a first o-ring is connected to the first end of the rod and the first end of the rod is inside an inner channel of the shaft of the impeller assembly.
29. The bioprocess vessel of claim 28, wherein the impeller assembly is freely rotatable against the rod.
30. A bioprocess vessel, comprising:
- a vessel body comprising a top portion and a bottom portion;
 - one or more necked access ports;
 - an impeller assembly comprising a shaft and a plurality of planar blades extending from the shaft;
 - a rod comprising a first end and a second end; and
 - a lever that extends through one of the one or more necked access ports;
- wherein the impeller assembly shaft is connected to the first end of the rod, the rod extends through a first screw comprising a reversible connection fitting for a second screw attached to an interior surface of the top portion of the vessel body at the center of the interior surface, and an o-ring reversibly connects the second screw to the second end of the rod;
- wherein the impeller assembly is partially retractable; and
 - wherein the bioprocess vessel is configured for activating and expanding T-cells.
31. The bioprocess vessel of claim 30, wherein the shaft comprises at least one hole that the lever can fit within.

32. The bioprocess vessel of claim 31, wherein rotating the lever in the at least one hole in a first direction disconnects the first screw from the second screw to extend the impeller assembly and rotating the lever in a second direction connects the first screw with the second screw to retract the impeller assembly.
33. The bioprocess vessel of claim 30, wherein the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-sixth and about two-thirds of the height of the vessel body.
34. The bioprocess vessel of claim 33, wherein the impeller assembly is configured to retract from a fully extended position up to a position that is between about one-third and about one half of the height of the vessel body.
35. The bioprocess vessel of claim 30, wherein the impeller assembly further comprises a magnet.
36. The bioprocess vessel of claim 35, wherein no magnetic interference occurs with magnetic beads when the impeller assembly is in a maximumly retracted state.
37. The bioprocess vessel of claim 30, wherein a first o-ring is connected to the first end of the rod and the first end of the rod is inside an inner channel of the shaft of the impeller assembly.
38. The bioprocess vessel of claim 37, wherein the impeller assembly is freely rotatable against the rod.
39. A method for activating and expanding T-cells, comprising the steps of:
- (a) providing a bioprocess vessel of any of the preceding claims;
 - (b) providing an aqueous solution to the bioprocess vessel that is suitable for T-cell activation and expansion;
 - (c) providing beads to the bioprocess vessel, wherein the beads comprise a T-cell activator;
 - (d) providing T-cells to the bioprocess vessel; and

(e) culturing T-cells activated by the beads in the bioprocess vessel.

40. The method of claim 39, wherein steps (a) and (b) are performed before step (c), and wherein step (d) is performed after step (c).

41. The method of claim 39, wherein steps (a) and (b) are performed before step (d), and wherein step (c) is performed after step (d).

42. The method of claim 39, further comprising step (f) mixing the aqueous solution with the impeller of the impeller assembly.

43. The method of claim 39, wherein the impeller comprises a magnet in a lower portion of the impeller.

44. The method of claim 39, wherein the T-cells provided to the bioprocess vessel are engineered T-cells.

45. The method of claim 44, wherein the engineered T-cells comprise a gene for a chimeric antigen receptor or for a T-cell receptor.

46. The method of claim 43, wherein the beads are magnetic beads, and wherein the magnetic beads do not attach magnetically to the impeller magnet during activation and expansion.

47. The method of claim 39, wherein the T-cell activator is an antigen to an $\alpha\beta$ -T-cell receptor on the T-cells.

48. The method of claim 47, wherein the antigen comprises anti-CD3 or anti-CD28, or a combination thereof.

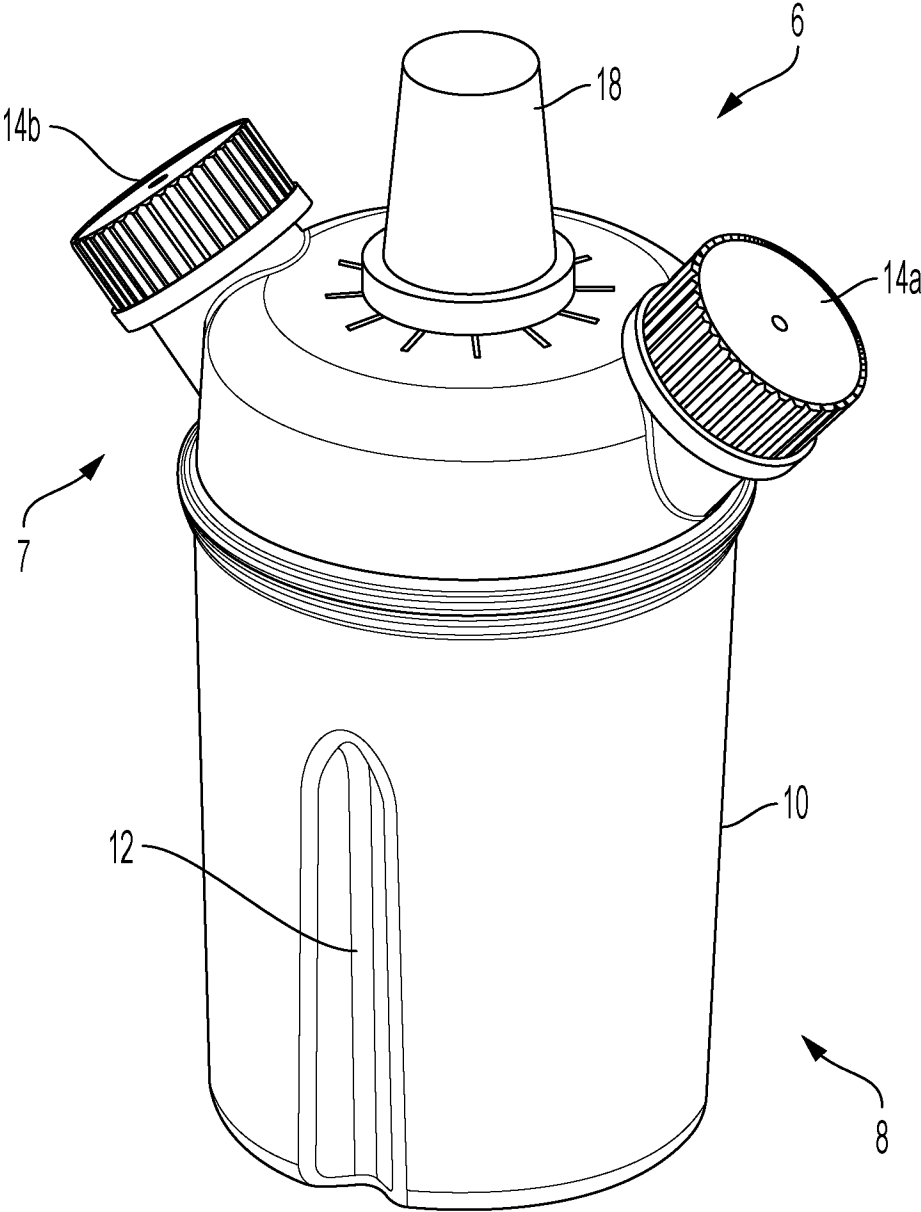


FIG. 1

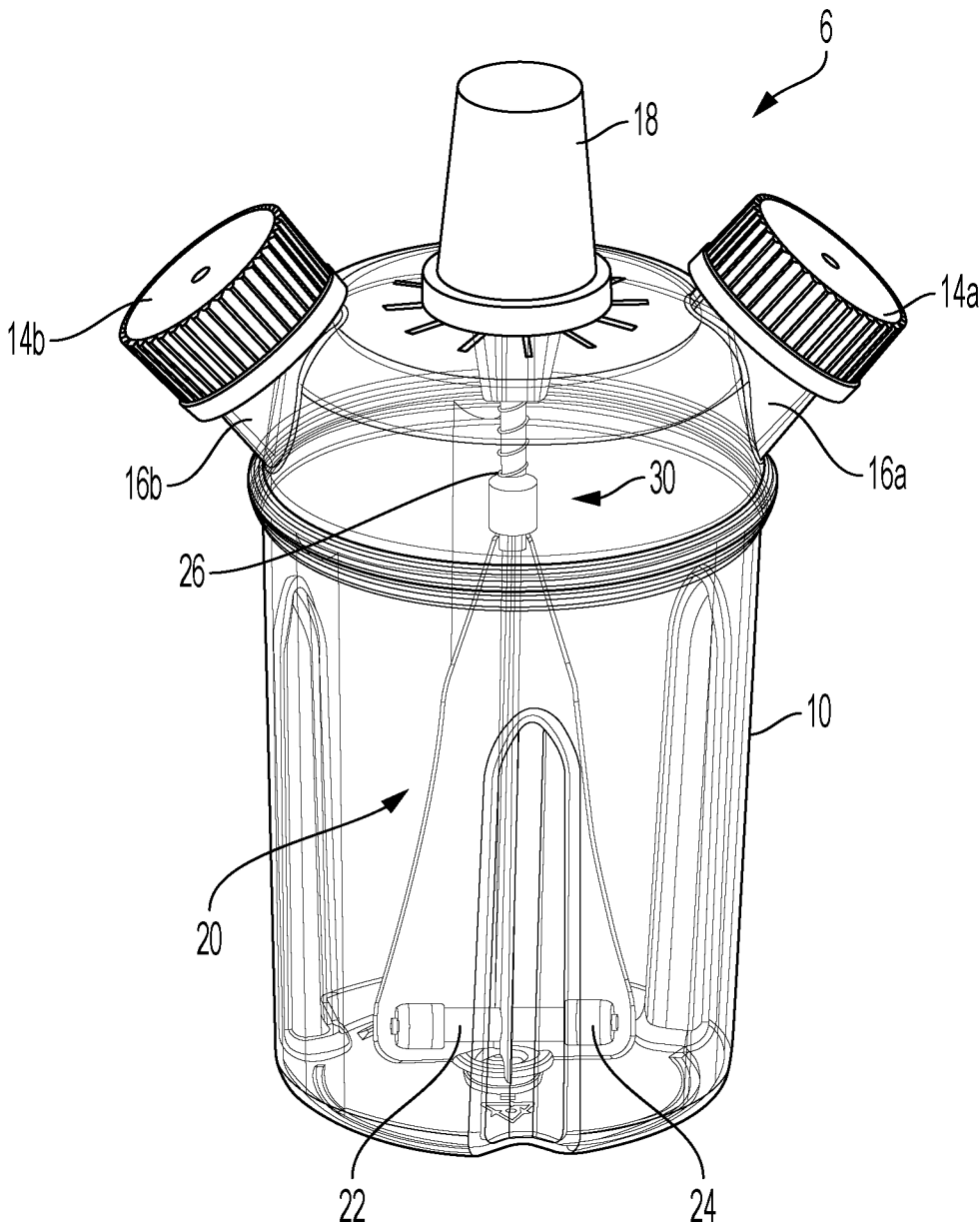


FIG. 2

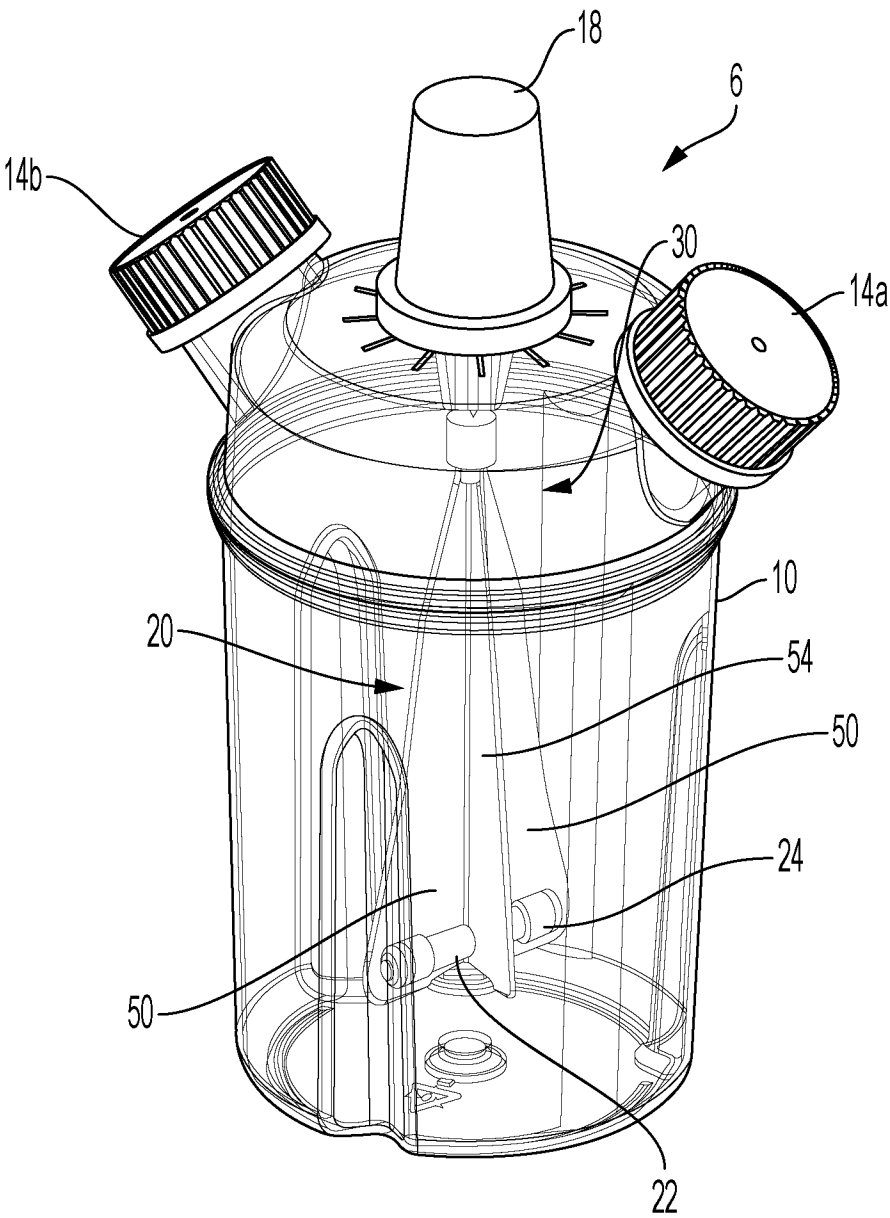


FIG. 3

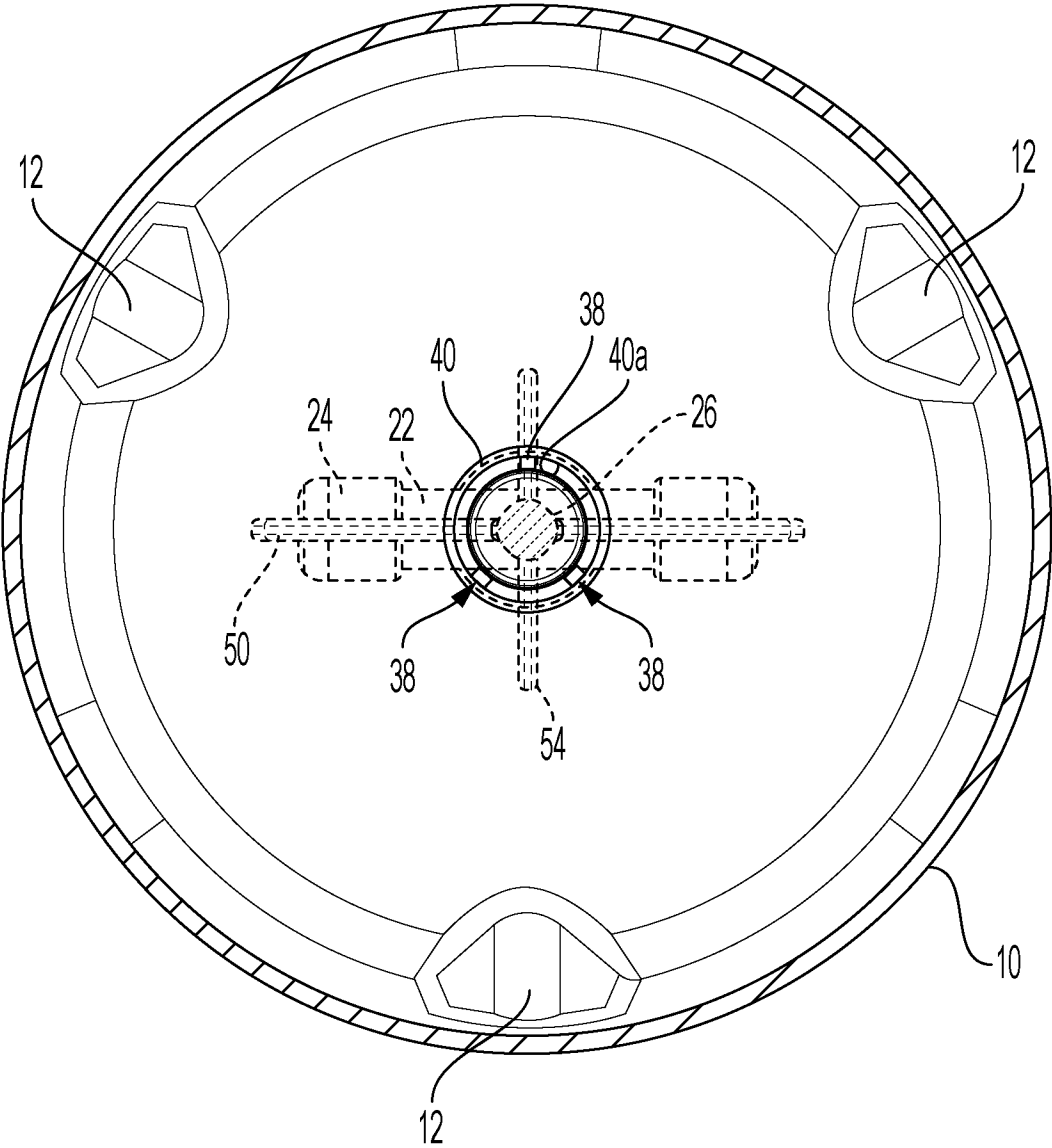


FIG. 4

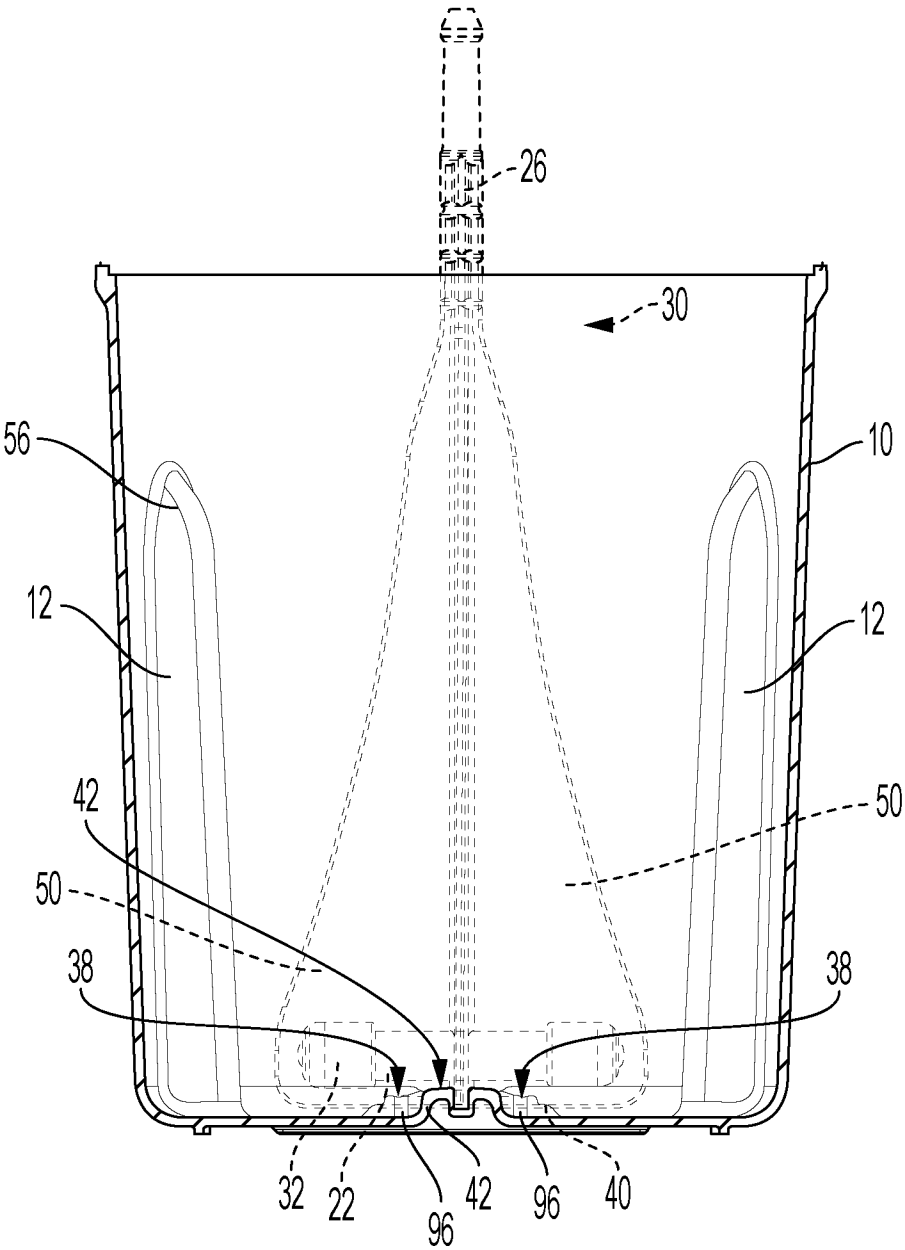


FIG. 5

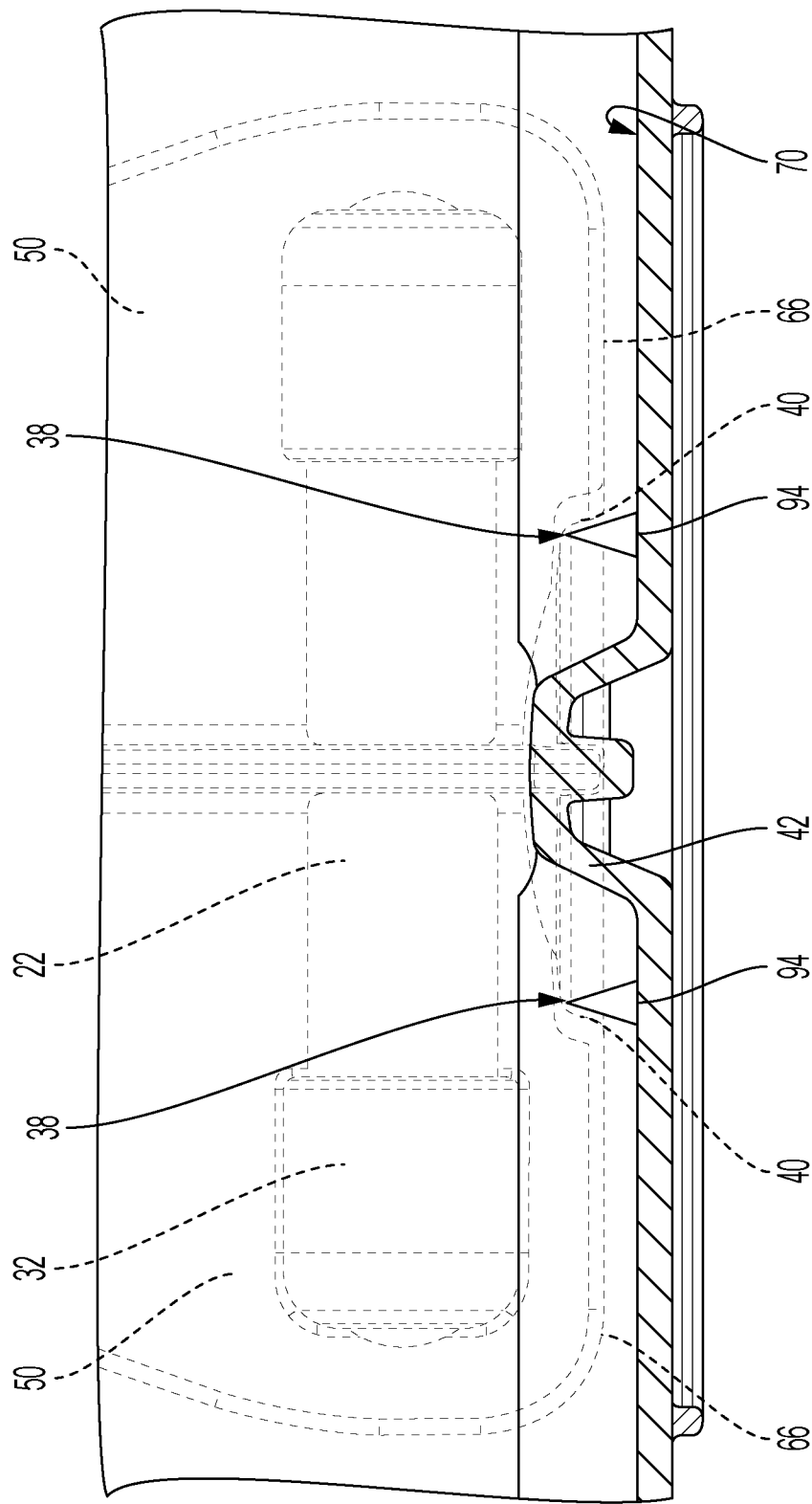


FIG. 6

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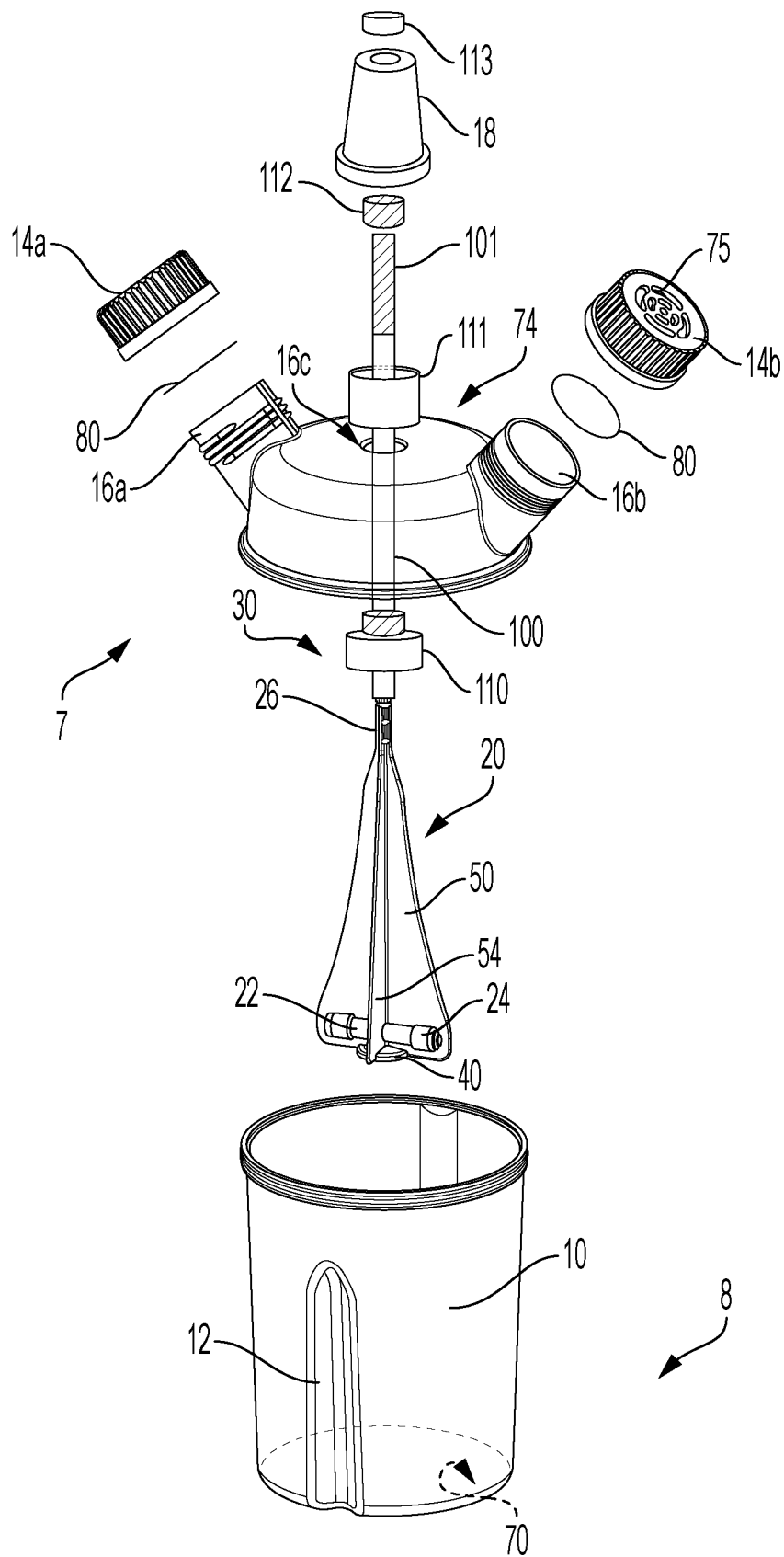


FIG. 7

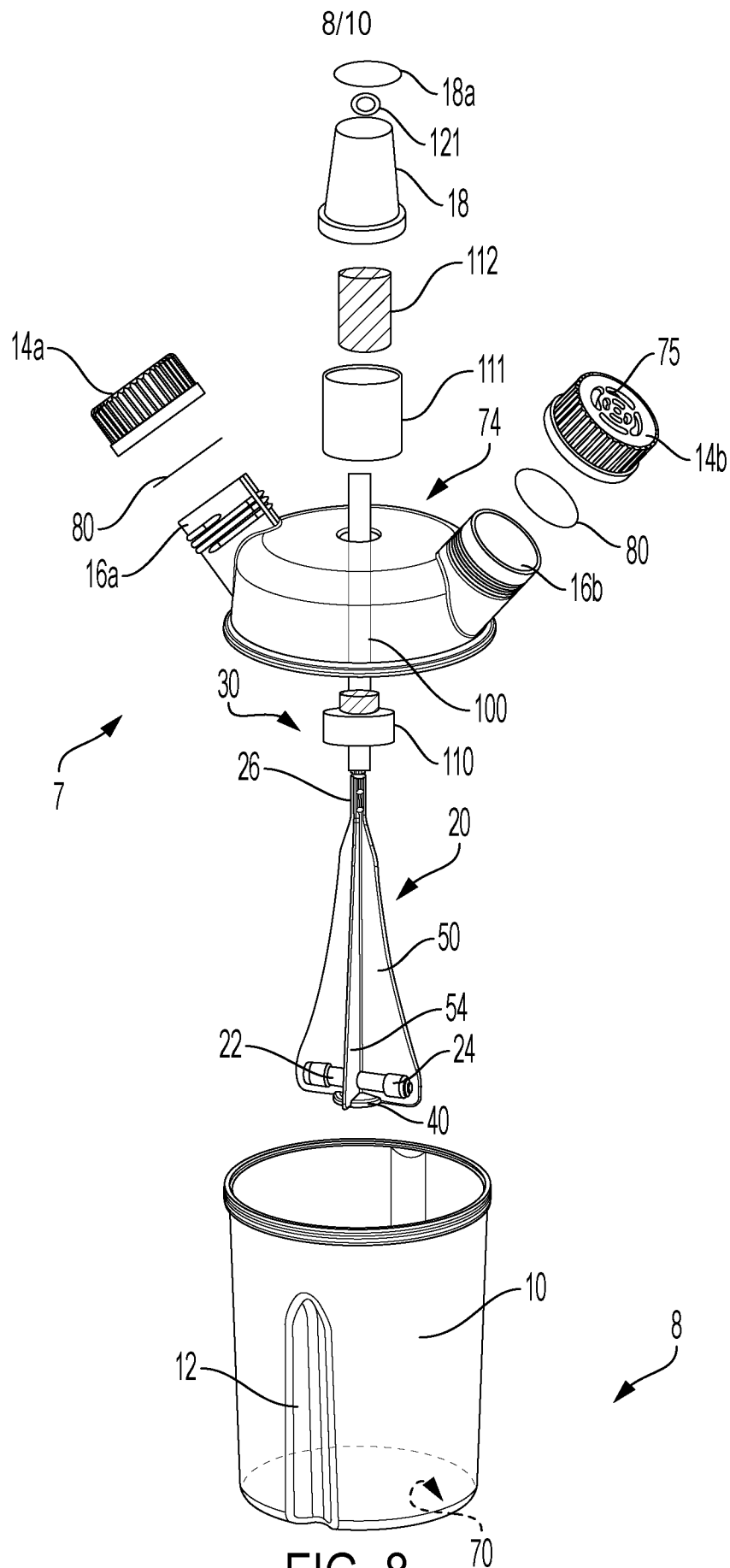
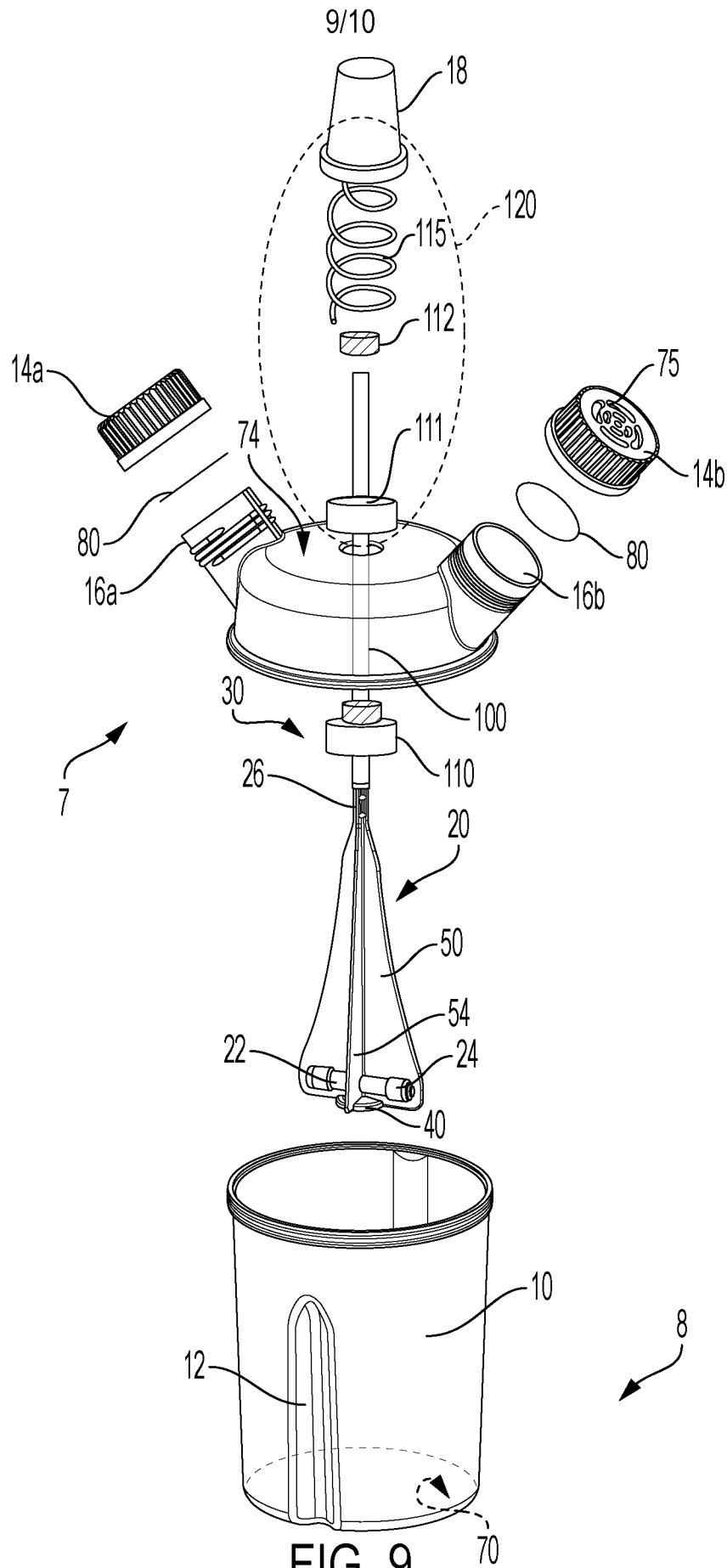


FIG. 8



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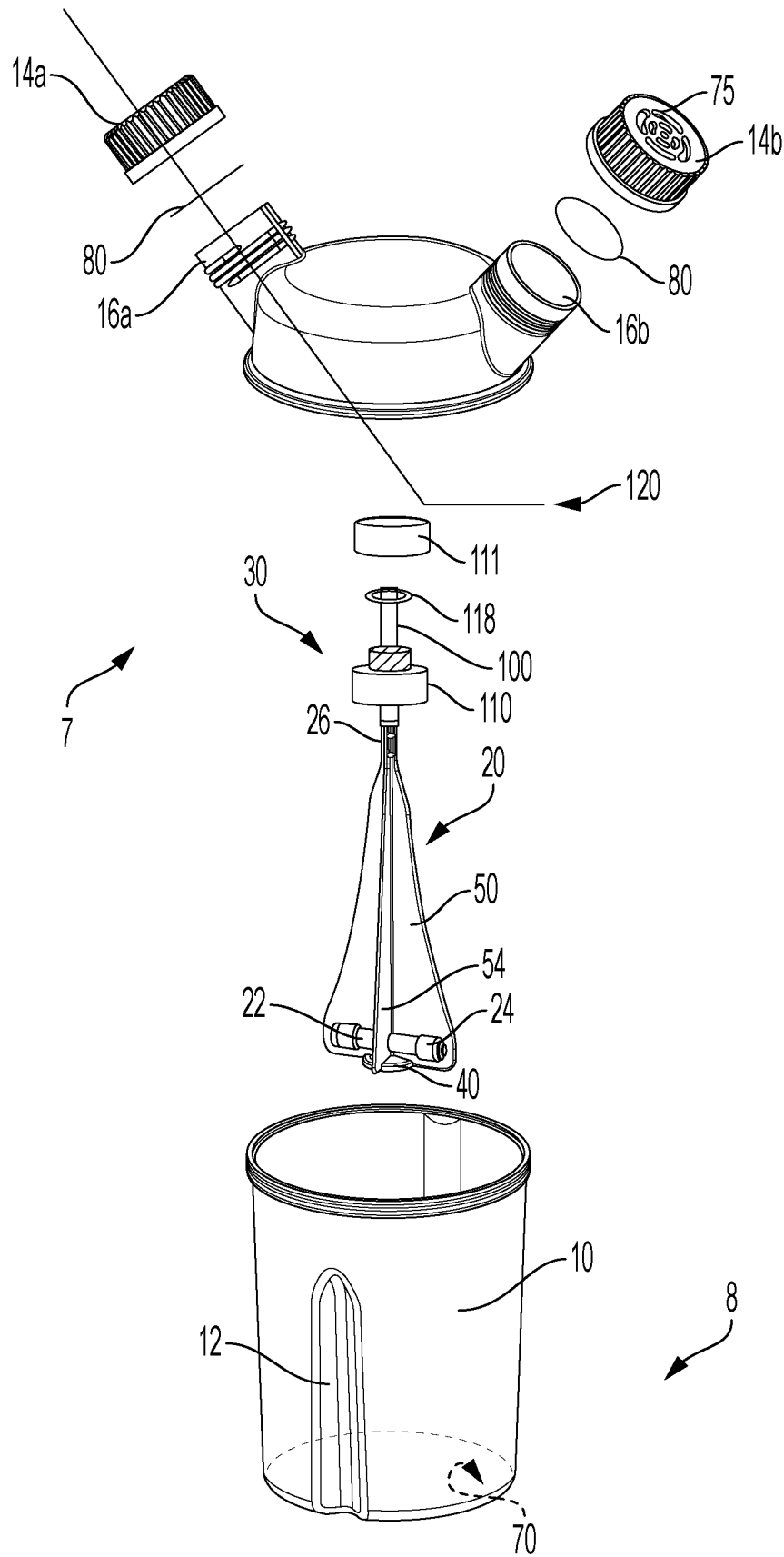


FIG. 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/055416

A. CLASSIFICATION OF SUBJECT MATTER INV. C12M1/00 C12M1/06 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) C12M		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data, BIOSIS		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 267 791 A (CHRISTIAN STEPHEN R [US] ET AL) 7 December 1993 (1993-12-07)	1-38
A	claim 1; figure 1 column 1, lines 6-14 column 2, lines 32-38 column 3, line 40 -----	39-48
A	US 2020/017830 A1 (SHI GUIXIN [US] ET AL) 16 January 2020 (2020-01-16) figures 1-4 -----	1-48
A	EP 3 464 550 B1 (CORNING INC [US]) 11 March 2020 (2020-03-11) claim 1; figure 1 ----- - / - -	1-48
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
<p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>		
Date of the actual completion of the international search 5 February 2025		Date of mailing of the international search report 18/02/2025
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Jones, Laura

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2024/055416

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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International application No

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