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(54) Title: METHOD AND APPARATUS FOR SELECTIVE DEPLETION OF BIOLOGICAL PARTICLES

(57) Abstract

A method and apparatus for effecting depletion of selected cells from a mixture of cells is disclosed. The device includes a plurality of vertically oriented binding chambers, each capable of containing biologically active receptor material such as polystyrene–fiber pads having "receptor" antibodies covalently bound to the fibers. A valve system is employed which allows the user of the device to route the cell mixture and rinsing fluids into and out of the binding chambers according to a preselected sequence. The flow of the cell mixture is such that it flows from the bottom of each chamber to the top of the chamber, pushed upward by hydrostatic pressure. Automatic cut–off means, such as hydrophobic valves, automatically terminate the flow of the cell mixture into the binding chambers when the level of the cell mixture reaches a predetermined point in the binding chambers. A rinse material, such as phosphate buffered saline, is selectively routed into the binding chambers from the top of the chambers. Optimal results are achieved by introducing the rinse fluid to the chambers at a specific flow rate. The first binding step is performed on cellular material of relatively high concentration and the second binding step is performed after substantially diluting the depleted cellular material produced during the first binding step.

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METHOD AND APPARATUS FOR SELECTIVE DEPLETION OF BIOLOGICAL PARTICLES

Field of the Invention

This invention relates to selective depletion or separation of one or more types of biological particles, for example, cells or virus particles, from a medium containing a mixture of different types of biological particles. In particular, the present invention concerns a method and apparatus for the separation of different types of biological particles based on proteins present on the surfaces of the biological particles.

The present invention will be described initially in connection with its use to separate one or more types of cells from a mixture comprising different cell types. However, there are other applications in which the invention can be used, as described below.

Cell depletion is a well known process by which one or more types of cells are removed or separated from a mixture of different cell types. There are two types of embodiments associated with cell depletion. One type is referred to as "positive selection" and the other type is referred to as "negative selection".

In an application involving positive selection, one or more "desired" cell types are isolated on a biologically active receptor (receptor) from a mixture of different cell types which include "unwanted" cells. The isolated cells are recovered from the receptor for use as desired and the mixture, depleted of the isolated cells and comprising principally the unwanted cells, is disposed of.

In an application involving negative selection, the isolated cells are the "unwanted" cells and the receptor with the unwanted cells is disposed of. The mixture of desired cells, depleted of the isolated "unwanted" cells, is used for its desired purpose.

The applications involving the use of cell depletion are many. For example, allogeneic transplantation in a patient of hematopoietic stem cells is accompanied frequently by serious patient complications due to the presence of immunocompetent T-cells in the transplant materials. The T-cells can attack host cells and cause Graft-vs-Host-Disease (GVHD). The incidence and severity of GVHD may be reduced by careful tissue matching of host and donor, but this reduces the likelihood of locating a successful donor by limiting the field of potential donors. Rather than attempting to find a perfect host-donor match, cell depletion based on negative selection may be used to pre-treat donated cellular materials to remove those cellular components associated with GVHD. Clinical and animal research related to allogenic transplantation of hematopoietic stem cells suggests that T-lymphocytes with CD5 and CD8 cell-surface protein markers are responsible for GVHD and that reduction of those cells will prevent or minimize GVHD.

It has also been found to be desirable to remove GVHD-causing cells from peripheral blood leukocyte collections intended for treatment of leukemic or lymphomatous relapse. In this type of application, it is believed that removal of cells with CD8 markers from the donor cells will prevent severe GVHD while allowing the remaining donor cells to aid in elimination of the malignant cells.

Reported Developments

Various techniques and apparatus are known for accomplishing cell depletion. Examples of such techniques include fluorescence cell sorting, the use of magnetic beads covered with antibodies, complement-mediated lysis, affinity chromatography, and centrifugal elutriation. There are disadvantages associated with the use of each of these techniques, for example, contamination by antibody or cell

lysis products, limited depletion of target cells, and adverse effects on non-target cells.

Certain techniques for effecting cell depletion involve the bonding to a substrate of a biologically active receptor which is capable of binding specifically to a complementary molecule and then exposing to the receptor a biological medium comprising a mixture of different cell types, including a cell type comprising the complementary molecule. The cell type comprising the complementary molecule binds to the receptor and is separated from the other cells comprising the biological medium. A few examples of receptors include a ligand which includes both haptens and antigens and a steroid-binding protein.

A widely studied technique for cell depletion involves the use of polystyrene. Polystyrene panning of cells was developed originally by Wysocki and Sato, P.N.A.S., 75:2844 (1978), utilizing passively adsorbed antibody on polystyrene. Unfortunately, only low recoveries can be achieved in the use of this technique and the process suffers from lack of specificity and contamination of the separated cells with antibody.

Other techniques which involve the use of polystyrene have been developed also to aid in cell depletion. They include containers which house stacked receptor-bound polystyrene sheets or receptor-bound polystyrene fiber pads which are flooded with a biological medium and then rinsed with a horizontal flow of rinse medium to draw away any unbound cells. Serial depletion treatments have been performed in which the unbound cells are depleted for a second time in a container identical to that used in a first depletion treatment. Such techniques require significant operator intervention which involves rotation or inversion of the device during use.

The present invention provides improved means for separating one or more types of biological particles from a medium containing different types of biological particles.

Summary of the Invention

In accordance with the present invention, there is provided an apparatus for selective separation of specific types of biological particles from a biological medium containing a plurality of biological particle types including the specific types, by contacting the biological medium with a biologically active receptor, comprising temporary storage means, having a bottom and a top, for temporarily storing the biological medium in contact with the receptor; biological medium input means for inputting the biological medium to the bottom of the temporary storage means; rinse medium input means for inputting a rinse medium to the apparatus; and automatic cut-off means for terminating the flow of the biological medium to the temporary storage means when a predetermined level of the biological medium has entered the temporary storage means, wherein the flow of the biological medium fills the temporary storage medium from the bottom of the temporary storage means to the top of the temporary storage means.

In preferred form, the apparatus of the present invention comprises means for inputting the rinse medium to the top of the temporary storage means at a predetermined flow rate that maximizes the depletion of the specific types of biological particles from the biological medium. Also, in preferred form, the temporary storage means of the apparatus comprises a primary binding chamber and a secondary binding chamber, with the secondary binding chamber having a larger volume than the primary binding chamber, and the apparatus includes most preferably selection means for selectively linking the primary binding chamber to the rinse medium input means and the biological medium input means, and for selectively linking the secondary binding chamber to the primary binding chamber and the rinse medium input means.

A preferred form of the automatic cut-off means comprises vent/valve means at the top of said temporary storage means, the vent/valve means including fluid-responsive means responsive to the presence of fluid for terminating the flow of fluid into the temporary storage means. In a most preferred form, the fluid-responsive means

comprises a hydrophobic material, for example, VERSAPORE R V-200R.

A preferred form of the receptor comprises one or more polystyrene fibers that are needle punched to form a polystyrene fiber pad, the polystyrene fibers having a diameter such that a pad so formed has an average pore size of approximately 100 - 140 μM (for example, a fiber diameter of approximately 70 - 100 μM).

Another aspect of the present invention is the provision of a method for selective depletion of specific types of biological particles from a biological medium containing a plurality of biological particle types, wherein the specific types of biological particles are bound to a biologically active receptor upon exposure thereto. In preferred form the method includes contacting the receptor to the biological medium for a first time period; removing the biological medium from contact with the receptor; diluting the removed biological medium with a rinse medium; contacting the diluted biological medium to the receptor for a second time period; and removing the diluted biological material depleted of the specific types of biological particles.

There are numerous advantages associated with the practice of the present invention. An increased level of cell depletion is achieved over the methods and devices of the prior art. Further, very little operator action is required, decreasing the likelihood of process failure due to operator error. In addition, the present invention is a closed system, assuring the maintenance of a high level of sterility for the material being processed by the device. These and other advantages will be readily apparent by reference to the accompanying specification, drawings and appended claims.

Brief Description of the Drawings

FIG. 1 is a cross-sectional block diagram of a device according to the present invention, showing the connection of input fluids to the device in a "fill" position;

FIG. 2 is a perspective view of a device in accordance with the present invention;

FIG. 3 is a partially exploded perspective view of the device shown in FIG. 2;

FIG. 4 is a partially exploded perspective view of the device shown in FIG. 2;

FIG. 5 is front sectional view of the device shown in FIG. 2 of the present invention, taken through line 5-5 of FIG. 2;

FIG. 6 is a side sectional view of the device shown in FIG. 2, taken through line 6-6 of FIG. 5;

FIG. 7 is a top sectional view of the device shown in FIG. 2, taken through line 7-7 of FIG. 5;

FIG. 8 is a sectional view of a vent/valve of the device shown in FIG. 2, taken through line 8-8 of FIG. 2;

FIG. 9 is a cross-sectional block diagram of a device according to the present invention, showing the device in a "fill" position;

FIG. 10 is a cross-sectional block diagram of a device according to the present invention, showing the device in a first "rinse" position; and

FIG. 11 is a cross-sectional block diagram of a device according to the present invention, showing the device in a second "rinse" position.

Detailed Description of the Preferred Embodiment

The present invention utilizes a receptor for effecting cell depletion or separation of other biological particles.

The term "receptor", as used herein, refers to a molecule which is capable of binding specifically to a complementary molecule comprising a biological particle. The type of receptor used in the present invention will vary according to the nature of the purification being performed. Antibodies, especially monoclonal antibodies, are particularly useful as receptors in the practice of the invention. Examples of other receptors include: cell surface membrane proteins which bind specifically to complementary molecules such as, for example, T-cells and hormones, including, for example, insulin; molecules which are found intracellularly such as, for example, steroid-binding proteins; molecules which are found in body fluids such as,

for example, thyroxine-binding globulin or lipoproteins; and a ligand which includes both haptens and antigens.

In preferred form, the receptor comprises fibers of polystyrene which contain functional groups that are capable of binding selectively to the surface of a biological particle, such as, for example, a cell surface protein or viral coat protein. The functional groups are typically linked covalently to the polystyrene fiber. Results of testing of devices utilizing pads composed of fibers shows generally equivalent performance to flat polystyrene plate devices. The fiber pads are superior, however, in certain respects, e.g., more CD34+ cells are recovered after a CD5/8 depletion with a fiber pad than from a similar depletion performed with a flat polystyrene plate device. Further, it is easier to work with fiber-pad devices because they permit the use of smaller devices that take advantage of the high surface area of fiber pads in comparison to flat plates. In addition, processing time and associated steps are reduced.

In preparing fiber pad devices, pre-made polystyrene (DOW 685D, Dow Chemical Co., Midland, Michigan) fibers are shot into a box, where pads are formed as the polystyrene fiber is needle punched (felted) to tangle the fiber into a mass. Any number of fibers may be utilized; the procedure can be performed with as few as one fiber. Fibers ranging in size from about 70-100 μM (microns) in diameter can be used; in the preferred embodiment, the polystyrene fiber pad is fabricated from polystyrene fibers that are approximately 75-85 μM in diameter. After compression between two heated plates to form pads, the pads are die cut to any desired shape and are then compressed again between two heated pads.

Polystyrene pads fabricated in this manner have a fiber-to-air ratio of approximately 20% fiber to 80% air and have an average pore size of approximately 100-140 μM. A pad so constructed provides for optimal hydrodynamic flow for the purpose of the present invention.

When a mixture of different types of biological particles is exposed to receptors, complementary molecules present in the mixture bind with the receptors and are retained. In an application involving protein retention, the

bound separated particles can be released from the receptors by means available in the art and put to their intended use. In an application involving negative selection, the unbound molecules can be collected and utilized for their intended purpose and the bound particles can be disposed of.

FIG. 1 is a cross-sectional block diagram of the device 10 of the present invention. Device 10 comprises a fluid control valve 15 (e.g., a spool valve) that controls fluid communication between a primary binding-chamber 20, a rinse-fluid input port 25, and a secondary binding-chamber 30. Primary binding chamber 20 and secondary binding chamber 30 are temporary storage means for temporarily storing a fluid, e.g., a biological medium input to the device.

Automatic cut-off means for terminating the flow of a liquid (e.g., the previously mentioned biological medium) into the temporary storage means comprises vent/valves 35, 40, and 45, which are associated with primary binding-chamber 20, rinse-fluid input port 25, and secondary binding-chamber 30, respectively. An additional vent/valve 50 is formed at the bottom of fluid control valve 15. The vent/valves 35, 40, 45, and 50 allow the passage of air but will block the flow of fluid; in the preferred embodiment the vent/valves comprise hydrophobic valves.

A biological particle source 55 (e.g., leukopack) is connected to primary binding-chamber 20 via an input port 56, and a rinse fluid source 60 (e.g., phosphate buffered saline) is connected to the rinse-fluid port 25 via an input port 61. A collection container 65 is connected to the device via an output 66. As described in more detail below, the fluid control valve 15 is operable to channel the contents of the biological material source and the rinse fluid source to the various locations of the device at appropriate times.

The structure of the preferred embodiment is now described with reference to FIGS. 2-8. All directional references herein (e.g., top, bottom, left, right) are made with reference to the orientation of the device as shown in the front sectional view of FIG. 5.

FIG. 2 is a perspective view of the device of the present invention. The fully assembled device 10 comprises a

valve body 70 sandwiched between a primary binding-chamber body 100 on the right and a secondary binding-chamber body 120 on the left. A valve plunger 130 is slideably inserted into valve body 70.

Referring to FIGS. 3 and 5, primary binding-chamber body 100 is coupled to the right side of valve body 70 and includes a recess 102 and recess 108, vent/valve 35, vent/valve 40, input port 56, and input port 61.

Primary binding-chamber 20 is defined by the recess 102 and the side wall of valve body 70 against which primary binding-chamber body 100 abuts when primary binding-chamber 100 and valve body 70 are coupled to each other. In the preferred embodiment the recess 102 is formed in the shape of a parallelogram. Among other things, the parallelogram shape provides for an even flow of fluid into the chamber.

A biologically active receptor 103 (e.g., a polystyrene fiber pad having specific antibodies bonded thereto, partially shown in FIG. 3) is placed in the recess 102 before the coupling of primary binding-chamber body 100 to valve body 70. In the preferred embodiment the polystyrene fiber pad is cut to the shape of the recess to maximize the amount of receptor material present in the primary binding chamber 20.

At the top of recess 102 is a recessed pathway 104. The depth of recessed pathway 104 is approximately ½ the depth of recess 102. End 106 of recessed pathway 104 is positioned such that it coincides with horizontal passage 78 of valve body 70 when primary binding-chamber body 100 is coupled to valve body 70. So coupled, a path of fluid communication is formed between the valve chamber 74 (discussed more fully below) and the primary binding-chamber 20; this communication path goes from the valve chamber 74, through horizontal passage 78, into recessed pathway 104, and into primary binding-chamber 20.

Vent/valve 35 is positioned adjacent to the intersection of the recess 102 and the recessed pathway 104, on the right side-wall of primary binding-chamber body 100 as shown in FIGS. 2 and 5. The purpose of vent/valve 35 is to allow air to flow freely out of the primary binding-chamber 20 while it

is filling with fluid, but to stop the flow of fluid into primary binding-chamber 20 when it is essentially filled with fluid. As mentioned above, in the preferred embodiment, each of the vent/valves of the present invention can comprise a hydrophobic valve, an example of which is shown in detail in FIG. 8. As long as the hydrophobic valve is dry it will allow air to pass through to the outside of the device and, therefore, incoming fluid will continue to flow freely. When the fluid reaches the level at which the hydrophobic valve becomes moistened by the fluid, air can no longer travel through the valve, and fluid flow ceases. Since the primary binding-chamber 20 is filled from the bottom, placement of the vent/valve 35 at the top of the primary binding chamber 20 will assure that the vent/valve 35 will stay dry until the fluid fills the reservoir.

Input port 56 provides a path of fluid communication between the exterior of the device and the primary binding-chamber 20. As previously mentioned, input port 56 is typically connected to a biological particle source so that the biological particles therefrom can be moved into the device for treatment.

Rinse fluid port 25 comprises a recess 108 (FIG. 3) which, in combination with the side wall of valve body 70, forms what is essentially a "de-bubbling" chamber for incoming rinse fluid introduced to the rinse fluid port via input port 61. Vent/valve 40 provides a path out of the device for air to travel so that air bubbles cannot form in the rinse line.

A low point 110 of the recess 108 (FIGS. 3 and 5) is in alignment with horizontal passage 76 in valve body 70 when the primary binding-chamber body 100 is coupled to valve body 70. So coupled, a path of fluid communication is formed between the valve chamber 74 and the rinse fluid port 25; this communication path goes from the rinse fluid port 25, through horizontal passage 76, and into valve chamber 74.

FIG. 4 is a partially exploded perspective view of secondary binding-chamber body 120. Referring to FIGS. 4 and 5, secondary binding-chamber body 120 is coupled to the right

side of valve body 70 and includes recess 122, vent/valve 45, and output 66.

Secondary binding chamber 30 (FIG. 5) is defined by recess 122 and recess 88 of valve body 70 when valve body 70 is coupled to secondary binding-chamber body 120. In the preferred embodiment the volume of secondary binding chamber 30 is larger than the volume of primary binding chamber 20. This is accomplished by constructing the secondary binding-chamber body 120 to be wider than primary binding-chamber body 100, thereby allowing the recess 122 to be deeper than the recess 102. The applicant has determined that a volume ratio of at least 1:4 between primary binding chamber 20 and secondary binding chamber 30 yields a superior and more efficient capture of cells than prior art systems that perform serial depletion steps using identically-sized chambers. A biologically active receptor 109 (e.g., polystyrene fiber pads having specific antibodies bonded thereto, partially shown in FIG. 4) is placed in the recess 122 and 88 before the coupling of secondary binding-chamber body 120 to valve body 70. The biologically active receptor 109 is formed to snugly fit in the recess 88 and 122, thereby maximizing the amount of receptor material present within secondary binding-chamber 30.

In the preferred embodiment, a single pad is placed in the primary binding chamber 20 and a plurality of pads of the same size are placed into secondary binding chamber 30, the exact number of pads being placed in secondary binding chamber 30 being determined by the size ratio of chambers 20 and 30 (e.g., if the ratio is 1:4, four of the polystyrene pads will be placed into secondary chamber 30).

With the exception of the depth of recess 122, recess 122 is essentially identical to recess 102. At the top of recess 122 is a recessed pathway 124, having the same depth as pathway 104. End 126 of recessed pathway 124 is positioned such that it coincides with horizontal passage 82 of valve body 70 when secondary binding-chamber body 120 is coupled to valve body 70. So coupled, a path of fluid communication is established between valve chamber 74 and secondary binding chamber 30; this communication path goes

from the valve chamber 74, thorough horizontal passage 82, into recessed pathway 124, and into primary binding chamber 20.

Vent/valve 45 is positioned adjacent to the intersection of recess 122 and recessed pathway 124, on the left sidewall of secondary binding-chamber body 120 as shown in FIG. 5. The purpose of vent/valve 45 is identical to that of vent/valve 35, i.e., to allow the flow of air through the vent (and the chamber) and to stop the flow of fluid into the chamber when the fluid level reaches the top of the chamber.

Referring to FIGS. 5 and 6, valve body 70 comprises an interior cylindrical wall 72 defining valve chamber 74. Valve chamber 74 is a vertical elongate cylindrical bore; a plurality of horizontal passages 76, 78, 80, 82, 84, and 86 extend in a radial direction through the valve body 70 and into the valve chamber 74. A recess 88 (FIG. 4) forms a side wall of secondary binding chamber 30 when secondary binding-chamber body 120 is coupled to valve body 70. In a preferred embodiment, recess 88 is formed in the shape of a parallelogram. The horizontal passages 82 and 84 each provide a route for fluid communication between valve chamber 74 and secondary binding chamber 30 when the device is fully assembled.

Horizontal passage 127 (FIG. 5) is formed through the secondary binding-chamber body 120, at a location beneath the recess 122. Horizontal passage 127 is positioned such that it coincides with horizontal passage 86 of valve body 70 when the secondary binding-chamber body 120 is coupled to valve body 70. When so coupled, the coincident horizontal passages 86 and 127 form a path of fluid communication from the valve chamber 74 to the outside of the device. As can be seen from FIG. 4, in the preferred embodiment a filter housing is formed by filter housing bores 87 and 128 at the point at which horizontal passages 86 and 127 meet; this housing enables the insertion of a filter 129 to filter fluids passing therethrough. Filter 129 can comprise a 40 micron screen filter; filter 129 filters out particulate matter that may exist in the depleted fluid.

Referring to FIGS. 5 and 6, fluid control valve 15 further comprises valve chamber 74, bottom vent assembly 90, and valve plunger 130. Valve plunger 130 has a cylindrical outer surface and is slideably mountable into valve chamber 74 for longitudinal reciprocation therein. In actual use the valve plunger 130 should only be moved downward within valve chamber 74; if desired, means can be included to prohibit the movement of the plunger 130 in the upward direction.

Valve plunger assembly 130 comprises a flared handle 134, an upper selection-pin hole 136, a lower selection-pin hole 138, a vertical passage 140, a horizontal passage 142, an alignment slot 144, O-rings 146, 148, and 150, alignment slot 154, a vertical passage 156, a horizontal passage 158, and O-rings 160, 162, 164, and 166. The flared handle 134 gives a user of the device a grasping area for manipulating the valve plunger during use. The upper selection-pin hole 136 and lower selection-pin hole 138 pass radially through the valve plunger 130 perpendicular to the longitudinal axis thereof. As described below, the upper selection-pin hole 136 and lower selection-pin hole 138 are used, in connection with selection pins 137 and 139, to enable the valve plunger 130 to be "locked" in certain positions to provide certain fluid paths through the device.

Vertical passage 140 is situated between O-ring 146 and O-ring 148 and runs parallel to the longitudinal axis of the valve plunger 130. Vertical passage 140 is a U-shaped channel formed in an upper portion of valve plunger 130. Horizontal passage 142 is formed radially through valve plunger 130 perpendicular to the longitudinal axis thereof. The location of horizontal passage 142 is selected so that it intersects with the bottom of vertical passage 140, forming a path of fluid communication longitudinally along the valve plunger 130 and radially through the valve plunger 130. When the valve plunger 130 is properly aligned in valve chamber 74, a first fluid communication path is established between the left and right sides of valve body 70.

Vertical passage 156 runs parallel to the longitudinal axis of the valve plunger 130. Vertical passage 156 is a U-shaped channel formed in a lower portion of valve plunger

130. Horizontal passage 158 is formed radially through valve plunger 130 perpendicular to the longitudinal axis thereof. The location of horizontal passage 158 is selected so that it intersects with the top of vertical passage 156, forming a path of fluid communication longitudinally along the valve plunger 130 and radially through the valve plunger 130. When the valve plunger 130 is properly aligned in valve chamber 74, a second fluid communication path is established between the left and right sides of valve body 70.

The O-rings 146, 148, 150, 160, 162, 164, and 166 are seated in the valve plunger 130 in annular O-ring recesses (not shown) formed integrally with valve plunger 130 in a conventional manner. The O-rings maintain a substantially tight seal within valve chamber 74 to prevent the passing of any substantial amounts of fluid past the O-rings.

An upper alignment-pin aperture 92 and a lower alignment-pin aperture 94 are formed horizontally through the front wall of valve body 20 and into valve chamber 74.

Alignment slots 144 and 154 (FIGS. 5 and 6) are narrow channels formed in a central portion of valve plunger 130. The length of the alignment slots 144 and 154 is selected so that the longitudinal travel of valve plunger 130 is not affected by the insertion of alignment pins 137 and 139 as described below.

It is important to orient the valve plunger 130 in the valve chamber 74 so that the horizontal and vertical passages in the valve plunger 130 are properly alignable with the various horizontal passages in the valve body 70. Maintenance of proper alignment of the valve plunger 130 requires that it be prohibited from rotating about its longitudinal axis within valve chamber 74. To keep the valve plunger so aligned, alignment pins 145 and 155 (FIGS. 5-7) are inserted into upper alignment-pin aperture 92 and lower alignment-pin aperture 94, respectively. The length of the alignment pins 145 and 155 should be such that they extend into the valve chamber 74 and engage with alignment slots 144 and 154. The alignment pins should be secured in place in the alignment-pin apertures 92 and 94; for example, as shown in FIGS. 6 and 7, the inside surface of alignment-pin

apertures 92 and 94 is threaded and the alignment pins 145 and 155 have a mating thread formed on their outer diameter so that they can be tightened into place within the alignment-pin apertures 92 and 94. With the alignment pins 145 and 155 secured in place, the valve plunger can only move longitudinally in valve chamber 74.

FIG. 8 illustrates a typical embodiment of vent/valves 35, 40, 45, and 50. Specific reference is made to vent/valve 35 in the following paragraphs; it is understood, however, that the description herein applies equally to vent/valves 40, 45, and 50 unless otherwise set forth.

Each vent/valve comprises a retaining member 180 and a vent 184. Retaining member 180 can comprise a generally disk-shaped member having an opening 182 formed through the center thereof as shown. Vent 184 comprises a retaining ring 186 that retains a piece of vent material 188 therein. Vent material 188 should be a hydrophobic material, e.g., VERSAPORE R V-200R, available from Gellman Scientific.

A vent passage 190 is formed through the outer wall of primary binding-chamber body 100, adjacent to the intersection of recess 102 and recessed pathway 104. Vent passage 190 creates an opening between the area to be vented (e.g., primary binding chamber 30) and the outside of the device.

A vent/valve seat 192 is formed in the outer wall of primary binding chamber 30; as seen in FIG.8, vent valve seat 192 comprises a stepped circular bore formed by a first bore 194 and a second larger diameter, shallower, concentric bore 196. Both bores 194 and 196 are concentric with vent passage 190. Vent 184 is seated in vent/valve seat 192, and then retaining member 180 is secured to the outer wall of primary binding chamber 30, holding the vent 184 in place. Since the vent material 188 is hydrophobic, air can vent from the primary binding chamber 30 to the outside of the device via vent passage 190 and the opening 182. Once the vent material 188 becomes wet from fluid in the primary binding chamber 30, vent 184 can no longer pass air and the path from primary binding chamber 30 to the outside of the device is effectively closed.

To prohibit any fluid leakage from leaving the device through the bottom of valve chamber 74, vent/valve assembly 90 (FIG. 3, 5 and 6) is situated beneath valve chamber 74. While the vent/valve 50 of vent/valve assembly 90 is identical to the vent/valves 35, 40, and 45, a special housing is required to hold it in place, due to the larger size of the bore of valve chamber 74. As shown in FIGS. 5 and 6, vent/valve assembly 90 comprises a bottom vent housing 200 and a bottom vent retainer 206. Bottom vent housing 200 includes vent/valve seat 202 which is sized to hold vent/valve 50 snugly therein. Vent/valve seat 202 is formed identically to vent/valve seat 192. A vent passage 204 is formed in bottom vent housing 200 so that a vent path is formed through bottom vent housing 200.

Bottom vent retainer 206 is essentially identical to retaining member 180 (FIG. 8) and has a vent passage 208 formed therein that aligns concentrically with vent passage 204 of bottom vent housing 200; when bottom vent retainer 206 is coupled to bottom vent housing 200, and then both are attached to the bottom of valve body 70, a vent path for air displaced by valve plunger 130 is created. To prevent the accidental leakage of fluid through the bottom of the device, it is preferred that vent/valve 50 comprise a hydrophobic valve.

The operation of the present invention will now be described with reference to FIGS. 1 and 9 - 11. Although the material being depleted in this example is mobilized peripheral blood (leukopack), it is not intended to limit the use of this device or the method of depletion described herein to leukopack; this method/device can be used to deplete any biological material comprising a plurality of particulate biological matter.

Although not required, in the preferred embodiment the leukopack is first plasma reduced using, for example, a stock plasma expressor. The plasma reduced leukopack is transferred to the biological particle source 55 (e.g., a standard plasma bag). Preferably, the volume of biological particle source 55 is equal to the volume of primary binding chamber 20, e.g., 100 ml. If the amount of leukopack does

not equal the desired volume, it is diluted by adding rinse material, such as phosphate buffered saline, until the desired volume is achieved.

Referring to FIG. 1, the leukopack now contained in biological particle source 55 is hung above the device 10, along with rinse fluid source 60. The rinse fluid source 60 should flow into the device at a rate of approximately 200 - 400 ml/minute; at a flow rate within this range, sufficient bonding of the complementary molecules to the receptor can take place. The applicant has found that a flow rate of 250 ml/minute is optimal; this can be achieved by using a 2 liter bag of phosphate buffered saline as rinse fluid source 60, connecting the bag to inlet 64 using tubing having a 1/4 inch inside diameter, and hanging the bag so that the distance D, from the outlet 62 of rinse fluid source 60 to inlet 61, is approximately 12 inches. So configured, a sufficient hydrostatic head pressure exists to rinse the biologically active receptor (e.g., polystyrene pads) at the desired flow rate. Obviously parameters may be varied as long as the flow rate remains within the desired range.

With the valve plunger 130 in the "fill" position as shown in FIG. 9 (both selection pins 137 and 139 in place), the material in the biological particle source 55 (e.g.,leukopack) gravity-feeds into the primary binding chamber 20, feeding up into the chamber from the bottom. The primary binding chamber 20 fills until the fluid reaches vent/valve 35 near the top of the chamber. Upon reaching the vent/valve 35, the fluid-flow from the leukopack bag stops because vent/valve 35 closes and prevents the displacement of any additional air or fluid in primary binding chamber 20. If desired, the leukopack bag can be removed from the device by heat-sealing the inflow tube and removing the bag. By heat sealing the inflow tube before removal of the bag, the sterile environment within the device 10 is maintained.

The material contained in primary binding chamber 20 is then allowed to incubate for a set period of time, for example, 30 minutes. This gives the biologically active receptor material in primary binding chamber 20 sufficient time to bind with the unwanted (complementary) cells.

At the end of the first incubation period, the lowermost selection pin 139 is removed from the spool valve 15, and the valve plunger is depressed to a first "rinse" position as shown in FIG. 10. Movement of the valve plunger to this first rinse position routes the rinse fluid connected to input port 61 to the top of primary binding chamber 20; the path of flow goes through horizontal passage 76, down vertical passage 140, through horizontal passage 78, and into primary binding chamber 20. At the same time, the bottom of primary binding chamber 20 is routed to the bottom of secondary binding chamber 30; the path of flow goes through horizontal passage 80 and horizontal passage 158, down vertical passage 156, through horizontal passage 84, and into second binding chamber 30. The rinse fluid flows through primary binding chamber 20 and into the bottom of secondary binding chamber 30, taking with it those cells that have not been bound by the biologically active receptor material contained in primary binding chamber 20. As with primary binding chamber 20, secondary binding chamber 30 fills from the bottom to the top with the mixture of rinse fluid and leukopack, and this continues until the fluid reaches the vent/valve 45 near the top of secondary binding chamber 30.

Upon termination of the flow of fluid into secondary binding chamber 30, a second incubation period takes place (e.g., 60 minutes). In secondary binding chamber 30 the cell materials are more than 4 times more dilute than they were when they entered the primary binding 20, since a substantial amount of rinse fluid is added to the initial 100 ml that initially filled primary binding chamber 20. In addition, the ratio of receptors to complementary molecules is substantially increased since the number of polystyrene pads is also greater in secondary binding chamber 30 and because there have already been a quantity of the complementary molecules removed in primary binding chamber 20.

Once the second incubation period is completed, the upper selection pin 137 is removed from the valve plunger 130, allowing the valve plunger 130 to be moved to the second and final "rinse" position as shown in FIG. 11. In this position, the rinse fluid bag is now directly connected to

the top of secondary binding chamber 30; the path of flow goes through horizontal passage 76, down vertical passage 140, through horizontal passage 142 and horizontal passage 82, and into secondary binding chamber 30. At the same time, the collection container 65 is directly connected to the bottom of secondary binding chamber 30; the path of flow goes through horizontal passage 84, down vertical passage 156, through horizontal passage 86, and into collection container 65. Rinse material flows into the top of secondary binding chamber 30, rinsing the chamber and moving the depleted cell material out of secondary binding chamber 30 and into the collection container, following the aforementioned flow path. The collection container 65 is positioned below the device to assure the free-flow of collected material into the collection container. The collection container 65, now containing depleted cell material, can be sealed and the depleted cells can be used for transfusion or stored in an appropriate manner.

Although the drawings portray the present device as being constructed of opaque materials, it is understood that it may be desirable to have the ability to view the flow of materials as they travel through the device; thus, it would be obvious to construct the present invention from a transparent material, e.g. LEXAN, rather than an opaque material.

Further, while in the example given above the leukopack is pre-treated by using plasma-reduced leukopack, acceptable results can be obtained by inputting untreated leukopack.

In addition, the device of the present invention is not limited to a two-chamber device; additional chambers may be added if it is desired to further deplete the leukopack.

Further, the various components of the present invention can be coupled using any known coupling means such as screws or glue; since it is anticipated that the present invention will be utilized in a disposable form, glue is the preferred coupling means.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be readily apparent to

those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

CLAIMS

WHAT IS CLAIMED IS:

1. An apparatus for selective separation of specific types of biological particles from a biological medium containing a plurality of biological particle types including said specific types, by contacting said biological medium with a biologically active receptor, comprising:

temporary storage means, having a bottom and a top, for temporarily storing said biological medium in contact with said receptor;

biological medium input means for inputting said biological medium to the bottom of said temporary storage means;

rinse medium input means for inputting a rinse medium to said apparatus; and

automatic cut-off means for terminating the flow of said biological medium to said temporary storage means when a predetermined level of said biological material has entered said temporary storage means, wherein said flow of said biological medium fills said temporary storage medium from the bottom of said temporary storage means to the top of said temporary storage means.

2. An apparatus as set forth in claim 1 wherein said apparatus further comprises means for inputting said rinse medium to the top of said temporary storage means at a predetermined flow rate that maximizes the depletion of said specific types from said biological medium, for example, at a flow rate between 200 ml/minute and 400 ml/minute.

3. An apparatus as set forth in claim 1, wherein said temporary storage means comprises a plurality of serially-linkable, vertically oriented chambers.

4. An apparatus as set forth in claim 1, wherein said temporary storage means comprises a primary binding chamber and a secondary binding chamber, said secondary binding chamber having a larger volume than said primary binding chamber.

5. An apparatus as set forth in claim 4, wherein the volume of said secondary binding chamber exceeds the volume of said primary binding chamber by at least a 4-to-1 ratio.

6. An apparatus as set forth in claim 4 wherein said primary binding chamber and said secondary binding chamber each include a bottom and a top, said apparatus further comprising selection means for selectively linking the bottom of said primary binding chamber to the bottom of said secondary binding chamber.

7. An apparatus as set forth in claim 6, further comprising collection means couplable to said secondary binding chamber, wherein said selection means further comprises means for selectively linking said rinse medium input means to the top of said primary binding chamber and to the top of said secondary binding chamber, whereby in a first rinse position a path of fluid communication is established between said rinse medium input means and the top of said primary binding chamber and also between the bottom of said primary binding chamber and the bottom of said secondary binding chamber, and in a second rinse position a path of fluid communication is established between said rinse medium means and the top of said secondary binding chamber and also between the bottom of said secondary binding chamber and said collection means.

8. An apparatus as set forth in claim 7, wherein said selection means comprises a spool valve.

9. An apparatus as set forth in claim 4, further comprising selection means for selectively linking said primary binding chamber to said rinse medium input means and said biological medium input means, and for selectively linking said secondary binding chamber to said primary binding chamber and said rinse medium input means.

10. An apparatus as set forth in claim 9, wherein said selection means comprises a spool valve.

11. An apparatus as set forth in claim 1, wherein said automatic cut-off means comprises vent/valve means at the top of said temporary storage means, said vent/valve means including fluid-responsive means responsive to the presence of fluid for terminating the flow of fluid into said temporary storage means. 12. An apparatus as set forth in claim 11, wherein said fluid-responsive means comprises a hydrophobic material, for example, VERSAPORE R V-200R.

13. An apparatus for selective separation of specific types of biological particles from a biological medium, comprising:

receptor means for binding said specific types of biological particles thereto, said receptor means being fabricated to allow the flow of fluid therethrough at a rate of approximately 200 - 400 ml/minute;

temporary storage means for temporarily storing said biological medium in contact with said receptor means;

biological medium input means for inputting said biological medium to said temporary storage means;

rinse medium input means for inputting a rinse medium to said temporary storage means; and

automatic cut-off means for terminating the flow of said biological medium to said temporary storage means when a predetermined level of said biological material has entered said temporary storage means.

14. An apparatus as set forth in claim 13, wherein said receptor means comprises one or more polystyrene fibers that are needle punched to form a polystyrene fiber pad, said polystyrene fibers having a diameter such that a pad formed therewith has an average pore size of approximately 100 - 140 μM (for example, a fiber diameter of approximately 70 - 100 μM).

15. An apparatus for selective depletion of specific types of biological particles from a biological medium containing a plurality of biological particle types, wherein said specific types of biological particles are bound to a biologically active receptor upon exposure thereto, said apparatus comprising:

primary storage means for contacting said receptor to said biological medium for a first time period;

removal means for removing said biological medium from contact with said receptor;

dilution means for diluting said removed biological medium with a rinse medium;

secondary storage means for contacting said diluted biological medium to said receptor for a second time period; and

removal means for removing said diluted biological material depleted of said specific types of biological particles.

16. A method for selective depletion of specific types of biological particles from a biological medium containing a plurality of biological particle types, wherein said specific types of biological particles are bound to a biologically active receptor upon exposure thereto, said method comprising:

contacting said receptor to said biological medium for a first time period;

removing said biological medium from contact with said receptor;

diluting said removed biological medium with a rinse medium;

contacting said diluted biological medium to said receptor for a second time period; and

removing said diluted biological material depleted of said specific types of biological particles.

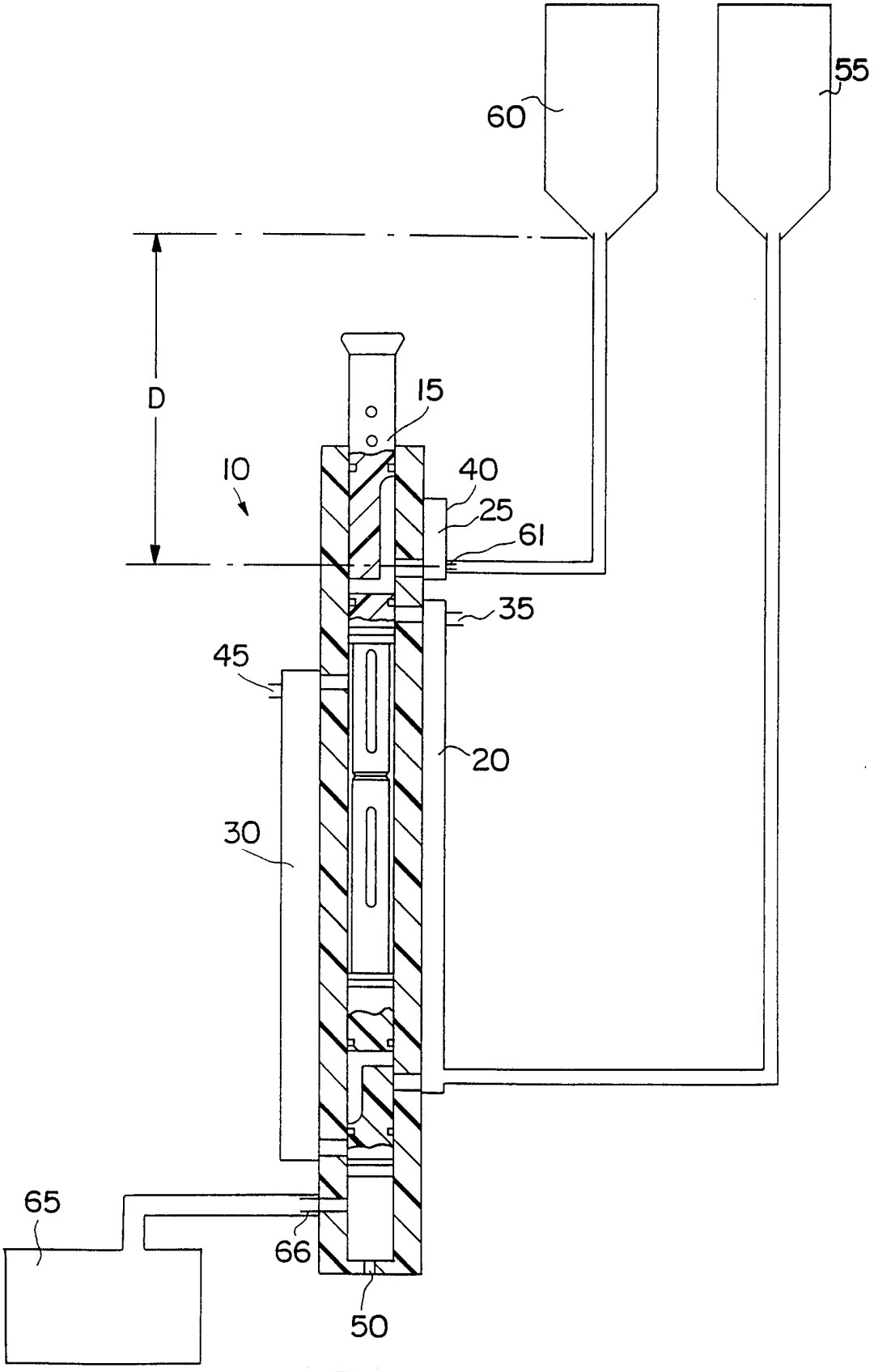

FIG. 1

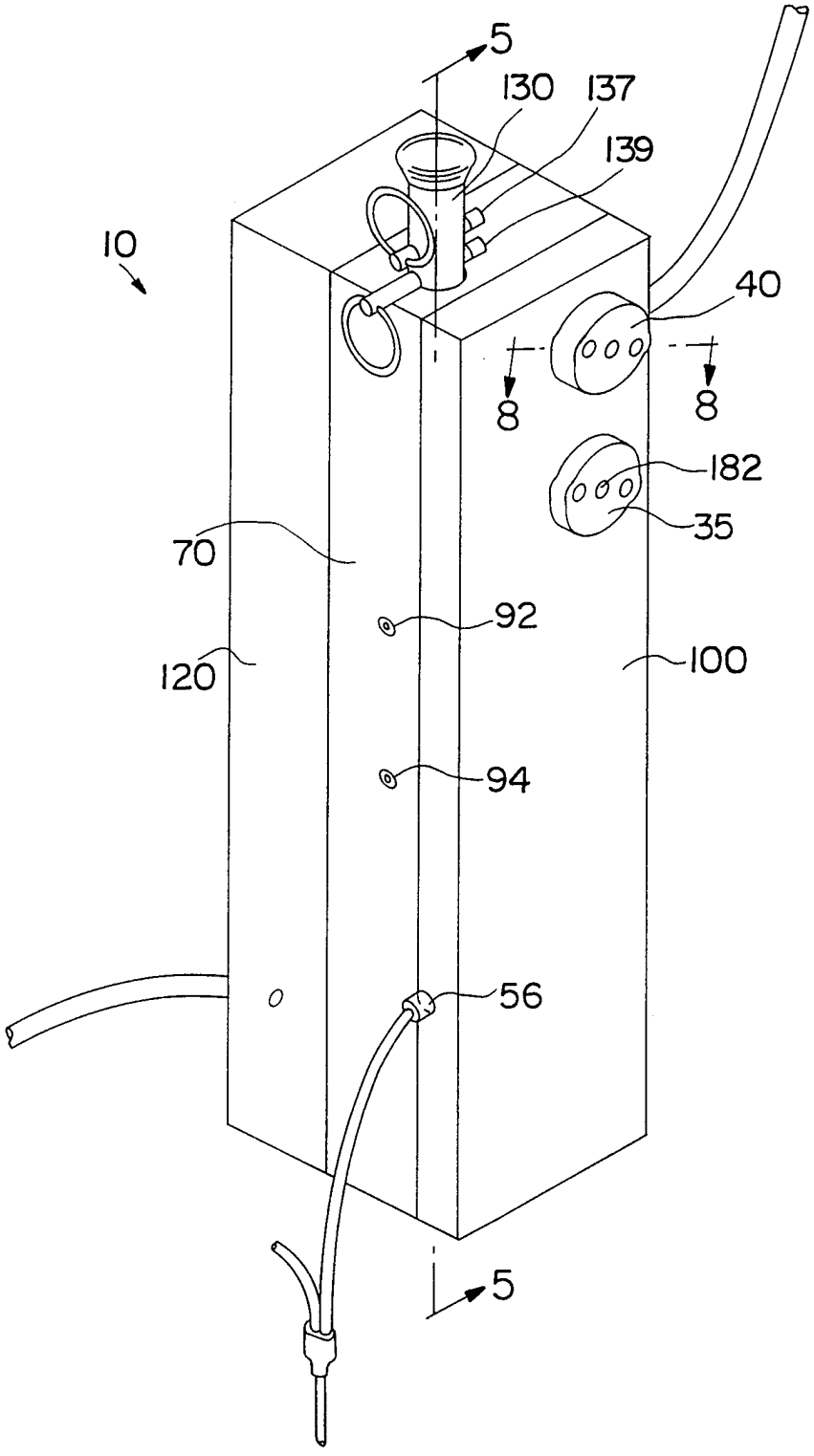

FIG. 2

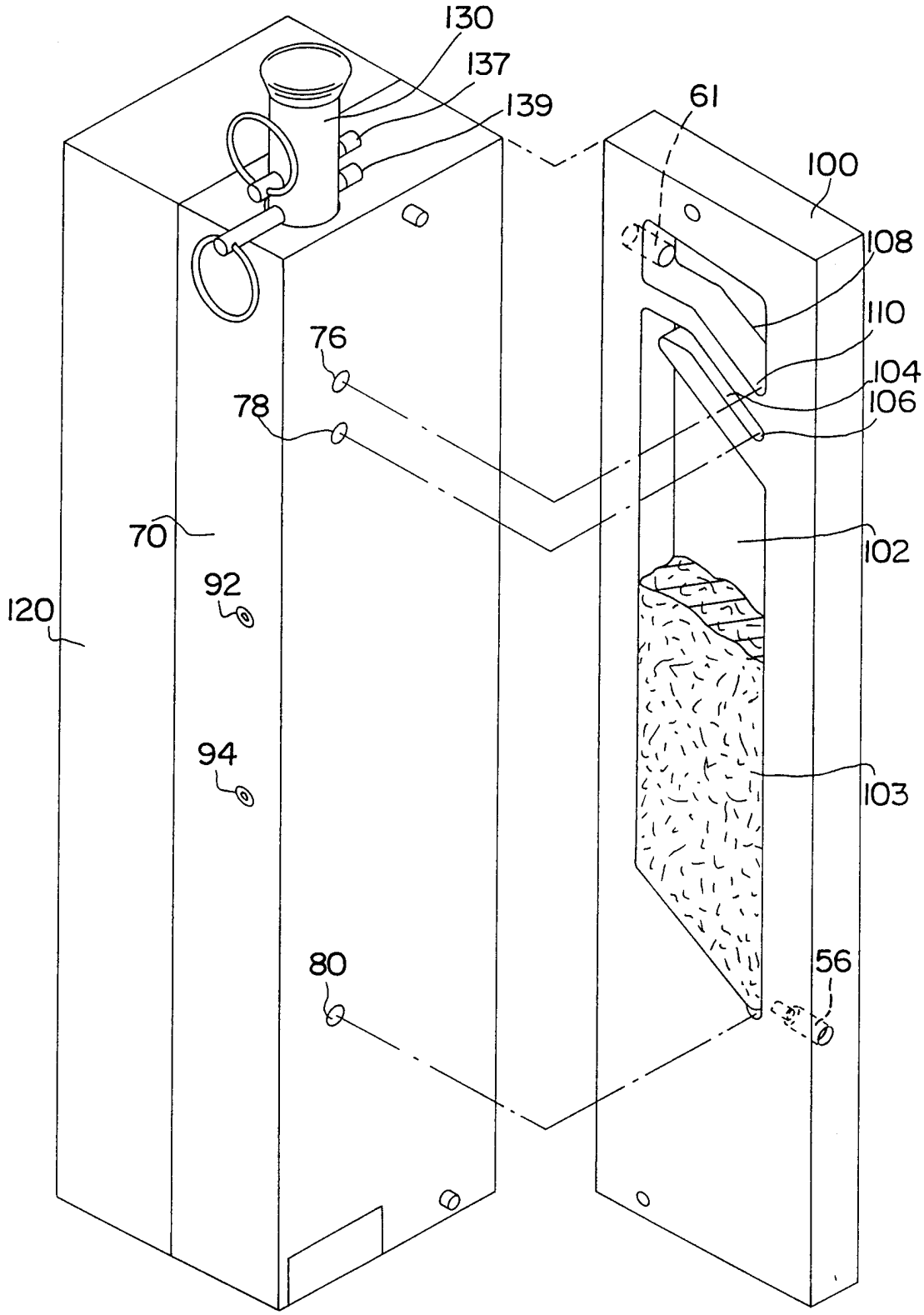

FIG. 3

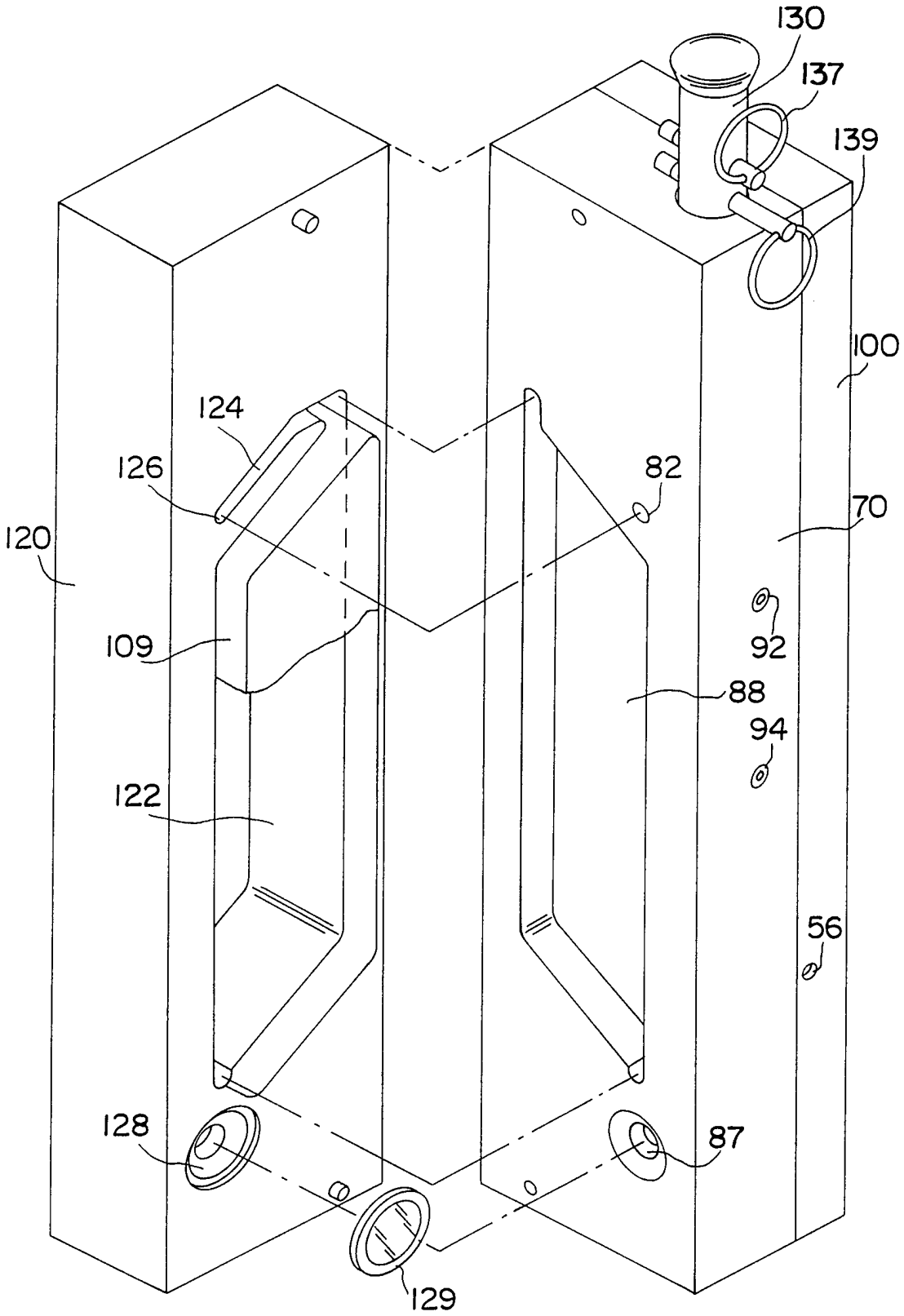

FIG. 4

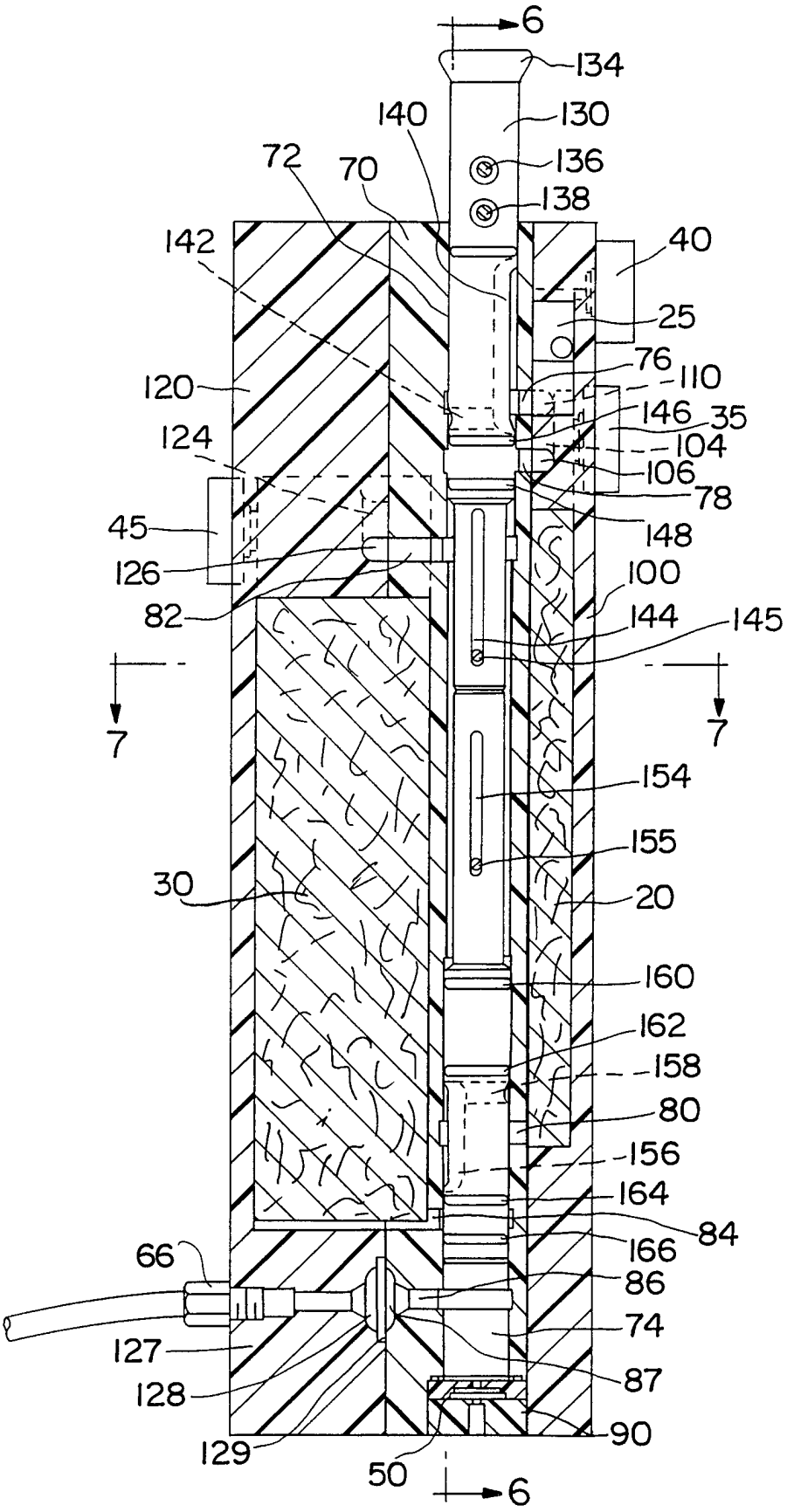
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FIG. 5

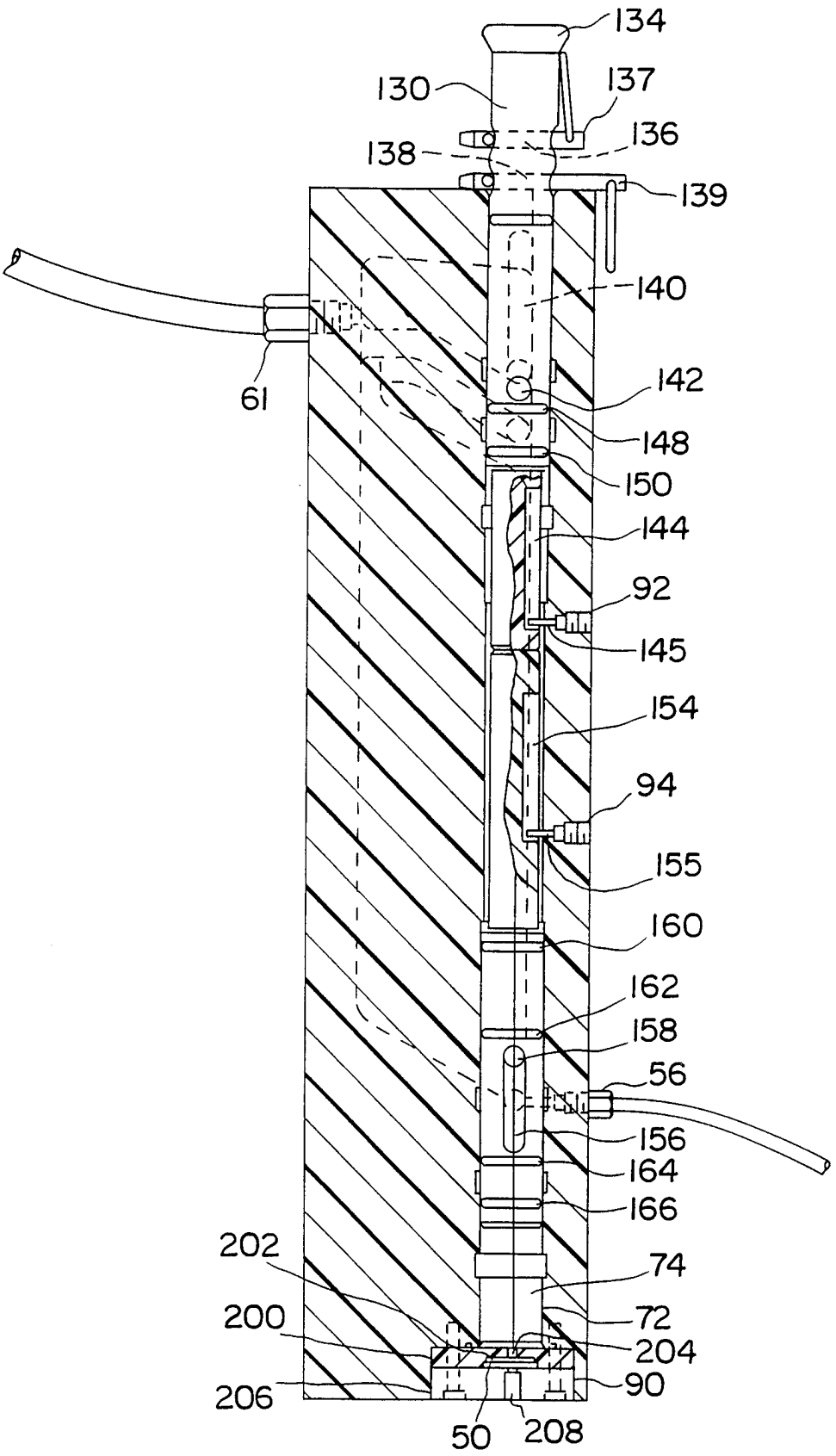
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FIG. 6

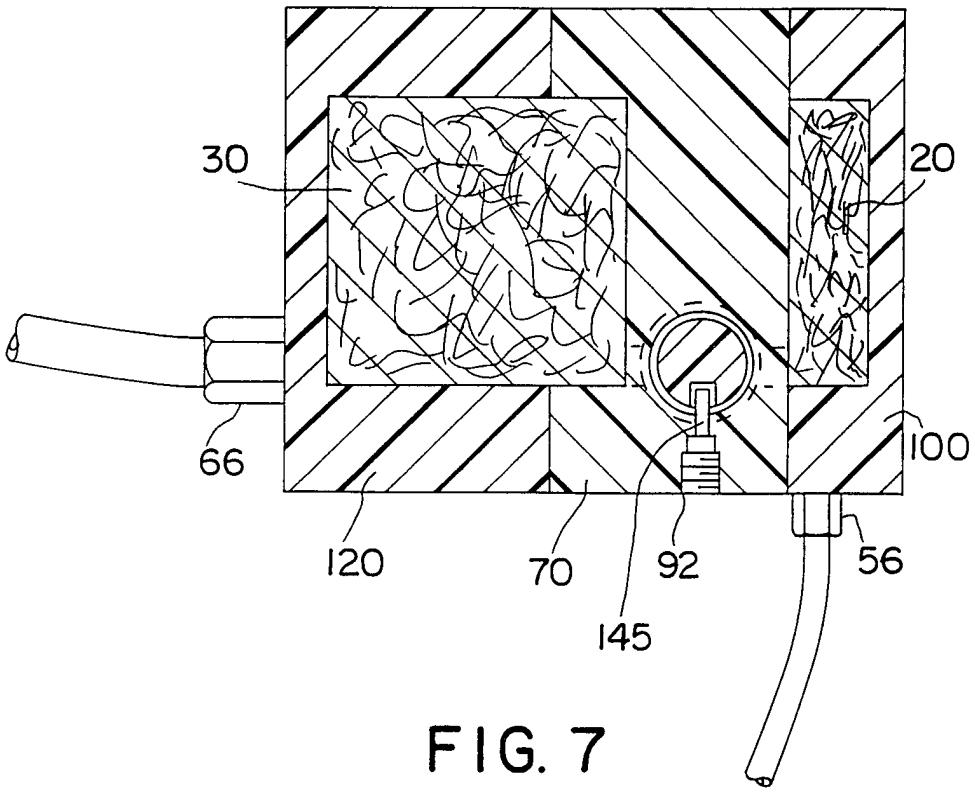

FIG. 7

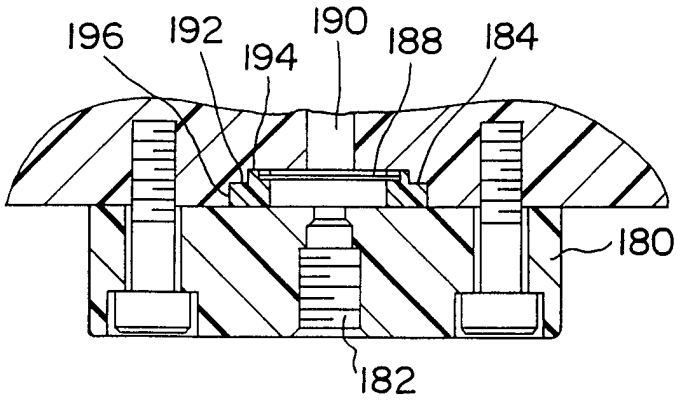

FIG. 8

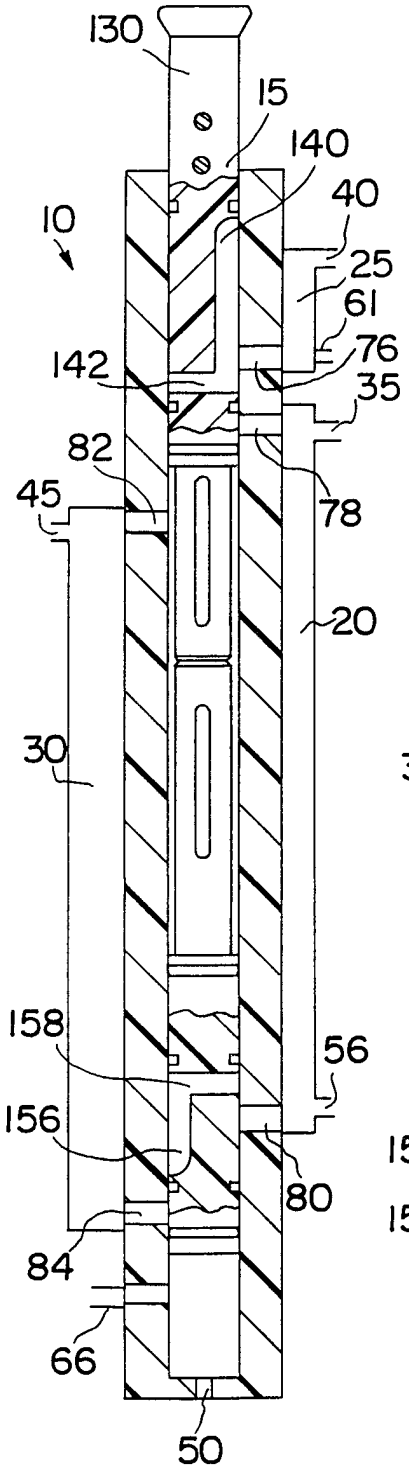

FIG. 9

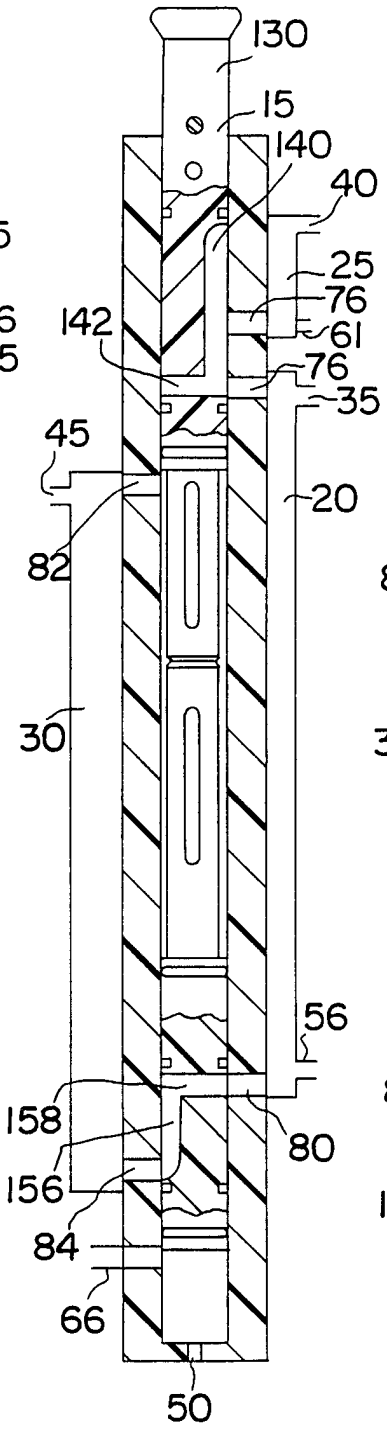

FIG. 10

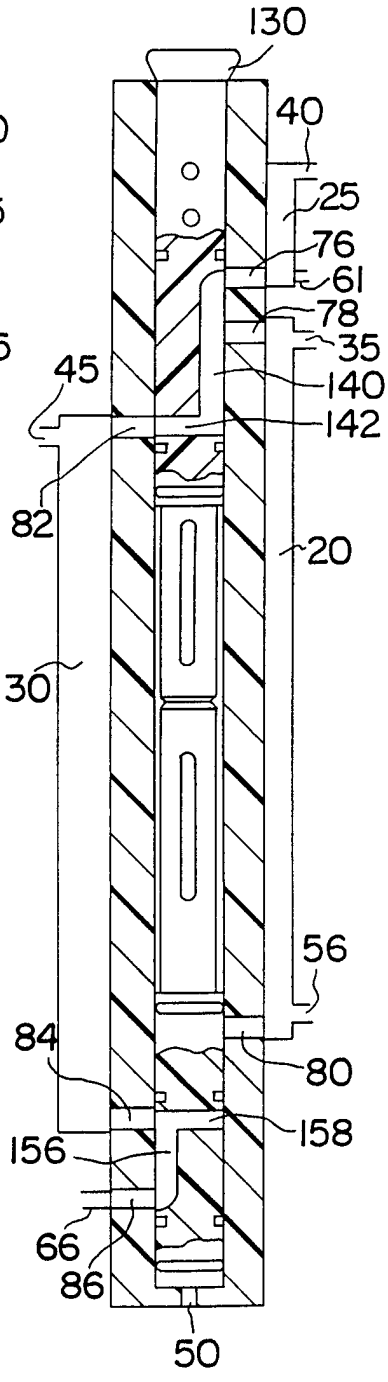

FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/23290

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :G01N 15/02, 15/10, 15/12, 33/53
US CL : 435/7.1, 286.1, 286.5; 210/97, 205; 422/99, 100
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)
U.S. : 435/7.1; 210/97, 205; 422/99, 100

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)
aps, biosis, caba, embase, lifesci, medline, scisearch

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, E	US 5,707,868 A (BOULAY ET AL.) 13 January 1998, Figures 1-3, col. 5, line 63 to col. 9, line 24.	1-16
Y	US 5,447,866 A (RUNYON) 05 September 1995, Figure 1, col. 3 line 30 to col. 7, line 53.	1-16
Y	US 5,316,905 A (MORI ET AL.) 31 May 1994, Figure 2A-2C, col. 5, line 11 to col. 21, line 3.	1-16
Y	US 5,246,855 A (KATINGER ET AL.) 21 September 1993, Figure 1, col. 3, line 25 to col. 6, line 29.	1-16
Y	US 5,183,740 A (LIGLER ET AL.) 02 February 1993, Figure 1 and 2, col. 5, line 20 to col. 14, line 28.	1-16

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier document published on or after the international filing date	"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
"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)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
24 MARCH 1998

Date of mailing of the international search report
27 APR 1998

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Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/23290

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 3,445,342 A (FREEDMAN) 20 May 1969 Figure 1.	1