



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p><b>(21) International Application Number:</b> PCT/US93/07216</p> <p><b>(22) International Filing Date:</b> 30 July 1993 (30.07.93)</p> <p><b>(30) Priority data:</b> 07/923,384 31 July 1992 (31.07.92) US</p> <p><b>(71) Applicants:</b> THOMAS JEFFERSON UNIVERSITY [US/US]; 11th and Walnut Streets, Philadelphia, PA 19107 (US). BECTON DICKINSON AND COMPANY [US/US]; One Becton Drive, Franklin Lakes, NJ 07417-1880 (US).</p> <p><b>(72) Inventors:</b> ALCHAS, Paul, G. ; 29 Ponds Circle, Wayne, NJ 07470 (US). PRAIS, Alfred, W. ; 245 Awosting Road, Hewitt, NJ 07421 (US). JARRELL, Bruce, E. ; 5700 N. Via Elena, Tucson, AZ 85718 (US). WILLIAMS, Stuart, K. ; 5131 N. Circulo Sobrio, Tucson, AZ 85718 (US). DIPISA, Joseph, A., Jr. ; 84 Maryann Lane, Wyckoff, NJ 07481 (US).</p>		<p><b>(74) Agents:</b> JOHNSON, Philip, S. et al.; Woodcock Washburn Kurtz Mackiewicz &amp; Norris, One Liberty Place, 46th Floor, Philadelphia, PA 19103 (US).</p> <p><b>(81) Designated States:</b> AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p><b>Published</b> <i>With international search report.</i> <i>With amended claims and statement.</i></p>
<p><b>(54) Title:</b> DEVICE AND METHOD FOR PROCESSING FAT TISSUE TO PRODUCE ENDOTHELIAL CELL PRODUCT</p>		
<p><b>(57) Abstract</b></p> <p>Methods and apparatus for collecting and processing tissue to produce an endothelial cell product having a vessel (11) for rinsing, draining, digesting and isolating tissue. The vessel (11) has a rinsing and digesting chamber (19) for containing tissue during processing. An inlet (20) in the rinsing and digesting chamber (19) allows entry of rinsing solution and tissue from a liposuction device. A waste chamber (22) in fluid communication with the rinsing and digesting chamber (19) preferably connects with a vacuum source. An isolation chamber (29) is separated from the rinsing and digesting chamber (19) by a screen (30). An ampule (31) in fluid communication with the isolation chamber (29) includes a pair of ports (34, 35) controlled by valve devices (37, 45) to be selectively in fluid communication with the isolation chamber (29). After processing, the ampule (31) isolates a pellet of endothelial cells and the valve devices (37, 45) permit the pellet to be in fluid communication with the ports (34, 35). The method includes providing the vessel (11), introducing tissue to be processed, orienting the vessel (11) to screen the tissue, introducing an enzyme and agitating to digest the tissue, centrifuging the vessel to transfer the cells from the digested tissue, and isolating the cells for retrieval.</p> <div style="text-align: right;"> </div>		

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**DEVICE AND METHOD FOR PROCESSING FAT TISSUE TO PRODUCE  
ENDOTHELIAL CELL PRODUCT**

**Cross-Reference to Related Applications**

5           This application is a continuation-in-part of  
application Serial No. 477,733, filed February 9, 1990 entitled  
"Device for Processing Fat Tissue to Produce Endothelial Cell  
Product," which is a continuation-in-part of application Serial  
No. 356,431, filed May 24, 1989 entitled "Endothelial Cell  
10 Procurement and Deposition Kit," which is a continuation-in-  
part of application Serial No. 244,496, filed September 12,  
1988 entitled "A Method of Treating a Synthetic or Naturally  
Occurring Surface with Microvascular Endothelial Cells and the  
Treated Surface Itself," which is a division of application  
15 Serial No. 742,086, filed June 6, 1985 and issued April 11,  
1989 as U.S. Patent No. 4,820,626 entitled "Method of Treating  
a Synthetic or Naturally Occurring Surface with Microvascular  
Endothelial Cells, and the Treated Surface Itself," each of  
which prior applications is assigned in whole or in part to  
20 Thomas Jefferson University, which is a co-assignee with Becton  
Dickinson and Company of the present application, which  
applications are hereby incorporated by reference.

          This application is also a continuation-in-part of  
application Serial No. 695,474, filed May 3, 1991 entitled  
25 "Device and Method for Collecting and Processing Fat Tissue and  
Procuring Microvessel Endothelial Cells."

          This application is related to applications Serial No.  
927,745, filed November 6, 1986 entitled "Method of Determining  
Endothelial Cell Coverage of a Prosthetic Surface"; Serial No.

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848,453, filed April 4, 1986 entitled "A Method of Treating a Synthetic or Naturally Occurring Surface with Collagen Laminate to Support Microvascular Endothelial Cell Growth and the Surface Itself"; Serial No. 114,242, filed October 28, 1987  
5 entitled "Method of Reendothelializing Vascular Linings", all of which are continuations-in-part of parent application Serial No. 742,086, filed June 6, 1985 and issued April 11, 1989 as U.S. Patent No. 4,820,626, each of which applications is assigned to Thomas Jefferson University, all of which  
10 applications are hereby incorporated by reference.

### Background of the Invention

While an autologous vein remains the graft of choice, advanced vascular disease and prior surgical intervention limit the availability of autologous grafts. Therefore, the use of  
15 synthetic grafts provides a means for restoring blood flow to ischemic areas when no alternative is available. Over the past three decades, artificial grafts have been used to provide immediate restoration of blood flow to areas of ischemia as a result of atherosclerotic vascular disease. In addition, they  
20 have been used to provide vascular access for hemodialysis in patients with chronic renal failure, and in the repair of arterial aneurysms.

Although initially successful in restoring perfusion to ischemic tissues, the long term prognosis for these grafts  
25 is not encouraging. Commercially available grafts are far from ideal due to their inherent thrombogenicity. Over an extended period of time, grafts less than 4 mm in diameter lose their patency as they become occluded via fibrin deposition and cellular adhesion. This process appears to be secondary, and  
30 to be due in part to the thrombogenic nature of the nude, i.e. non-endothelialized, surface of an implanted prosthesis. See Berger et al., "Healing of Arterial Prostheses in Man: Its Incompleteness", Ann. Surg. 175:118-27 (1972).

Thus, much current research is being focused on lining  
35 prostheses with human endothelial cells, for producing a non-thrombogenic endothelial cell surface such as exists in native

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human vessels. In dogs, seeding of endothelial cells onto both small and large diameter grafts has been shown to result in a complete endothelial cell lining in one to four months. Since vascular endothelium is said to represent a unique non-  
5 thrombogenic surface, endothelial cells are reported to be "the first logical choice for lining small diameter vascular grafts." The transplantation of a functional endothelial cell lining onto the surface of a vascular graft has proven to increase patency rates and decrease thrombus formation on the  
10 flow surface in animal models.

Past and present studies have focused on the isolation of large vessel endothelial cells from vein segments, with the subsequent seeding of these cells on the graft luminal surface. Tissue culture advances have also made the generation of large  
15 numbers of endothelial cells for high-density seeding on vascular prosthesis possible. These techniques have major drawbacks in the clinical setting. Endothelialization occurs at a slow rate, when low density seeding techniques are applied. High-density seeding, using cultured endothelial  
20 cells, requires the use of undefined media not easily applicable to the clinical setting.

It has been recognized that human microvascular endothelial cells i.e. the cells which are derived from capillaries, arterioles, and venule, will function suitably in  
25 place of large vessel cells even though there are morphological and functional differences between large vessel endothelial cells and microvessel endothelial cells in their native tissues. Microvascular endothelial cells are present in an abundant supply in body tissue, most notably in fat tissue, and  
30 may be used to establish a degree of implantation confluence prior to implantation, i.e. at least 50%, which dramatically improves the prognosis of most implants. For purposes of further description, fat tissue is designated as the exemplary source of microvascular endothelial cells, but it is recognized  
35 that endothelial cells from other tissues may be used as well.

To overcome the problems associated with seeding large vessel endothelial cells on prosthetic grafts, methods were

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developed for the isolation of microvessel endothelial cells from autologous adipose tissue, followed by high density seeding of a vascular prosthesis.

Although microvessel endothelial cells have been shown  
5 to be capable of endothelializing a blood-contacting surface, methods of procuring and depositing these cells in an operating room setting present special considerations. A vascular graft or other implant is treated to confluence using microvascular endothelial cells which are separated from fat, which is  
10 obtained at the beginning of the same uninterrupted surgical procedure. Fat tissue is removed from the patient after sterile conditions have been established. Microvascular endothelial cells in that fat area are then quickly separated from their related tissue by enzymatic digestion and  
15 centrifugation, and are used to treat a surface which is then implanted in the patient during the latter stages of the same operation. This procedure permits a patient to receive a graft which has been treated up to or above confluence with his own fresh endothelial cells.

20 The microvascular rich tissue obtained is perinephric fat, subcutaneous fat, omentum, or fat associated with the thoracic or peritoneal cavity. This tissue is then subjected to digestion using a proteolytic enzyme such as collagenase, comprising caseanase and trypsin, which is incubated with the  
25 tissue until the tissue mass disperses to produce a tissue digest. The microvascular endothelial cells are then separated from the digest using low speed centrifugation to produce an endothelial cell rich pellet. The pellet is washed with a buffered saline solution.

30 The resulting microvascular endothelial cells are then, preferably, suspended in buffered saline solution containing plasma protein, preferably about 1% plasma protein. This suspension, which comprises, on a volumetric basis, a pellet to solution ratio of 1:5 to 1:15, or preferably about  
35 1:10, is then used to treat the surface by incubating cells with that surface until sufficient adherence of the microvascular endothelial cells to that surface occurs to

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provide at least 50° confluence. As a result, an improved graft implant is provided having endothelialized surfaces which are either confluent, or which reach confluence quite rapidly (doubling within one population) following implantation.

5           Implants which can be treated to produce such an endothelial cell lining include but are not limited to, for example, intravascular devices such as artificial vascular prostheses, artificial hearts, and heart valves. The kit and methods of this invention for endothelializing surfaces can be  
10 used for surfaces comprised of known synthetic materials such as polyester, polytetrafluoroethylene (PTFE), or naturally occurring materials, such as umbilical vein, saphenous vein, and native bovine artery.

          Methods currently used employ standard laboratory  
15 equipment such as beakers, flasks, centrifuge tubes, shaker baths, pipettes, syringes, and sterile hoods. In one method the donated tissue is immediately transferred to ice cold buffered saline (pH 7.4) wherein the buffering agent is preferably a phosphate, i.e. a phosphate buffered saline (PBS).  
20 The tissue is minced with fine scissors and the buffer decanted. The proteolytic enzyme collagenase, containing caseanase and trypsin, is added to the tissue and incubated at 37 C until the tissue mass disperses. The digestion occurs within 30 minutes and generally should be less than 20 minutes.  
25 The digest is transferred to a sterile test tube and centrifuged at low speed (700 x g) in a table top centrifuge for 5 minutes at room temperature.

          The pellet of cells thus formed consists of greater than 95% endothelial cells. These endothelial cells are  
30 described herein as microvascular endothelial cells (MEC) since they originate from the arterioles, capillaries and venule, all elements of the microvasculature. The MEC pellet is washed 1 time by centrifugation with buffered saline, preferably PBS. The MEC suspension is then, preferably, pelletized by  
35 centrifugation (200 x g) and the pellet resuspended with protein containing buffer solution. Resuspension should be performed at a ratio of approximately 1:5 to 1:15 or about 1:10

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volumes of packed microvascular endothelial cells to buffer solution. The cell suspension is added to tubular grafts and the ends clamped, or the cells layered upon the surface to be treated.

5           Optimum periods for cell interaction vary upon the material of the prosthesis, the nature of any pretreatments it may have received, and whether the surface of the prosthesis has been modified to improve its acceptance of the MEC. Following incubation for a sufficient time to permit adherence  
10 of the endothelial cells with the prosthesis surface, the surface is washed with a protein containing buffer.

In United States Patent No. 4,820,626, and related applications, methods of treating a graft surface with endothelial cells are disclosed. According to those methods,  
15 subcutaneous adipose tissue is aspirated via a cannula and transferred by vacuum into a mucous trap. The trap is then transferred to a sieve inside a funnel which is placed in a sterile beaker. A rinsing solution is then poured over the tissue to remove red blood cells and lysed fat. The tissue is  
20 manually poured into a sterile Erlenmeyer flask containing collagenase solution and agitated at 37° C for 20 minutes. The collagenase slurry is manually poured into sterile conical centrifuge tubes, and spun for seven minutes at 700 x g. The endothelial cells are then pipetted out of the tube. A graft  
25 is tied to a male luer extension, and secured within a tube. The cells are resuspended in serum protein media and drawn into a syringe using a needle and syringe. The cells are forced into the lumen of the graft. The graft is manually rotated for 2 hours.

30           In spite of these advances, a need still exists for a simple, reliable method for producing endothelial cell coatings on a graft in an operating room setting, and this invention solves that need. The present invention provides for the ready isolation of large quantities of endothelial cells  
35 which can be easily performed in an operating room. While endothelial cells can be isolated from tissues other than fat, such as brain, lung, retina, adrenal glands, liver and muscle,

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the use of fat tissue as the source of the cells is preferred due to its abundance and availability, and due to the fact that its removal should not adversely affect the patient being treated. Although less preferred, it is possible to obtain  
5 human perinephric fat from brain-dead but heart beating cadaver donors, or from donors other than the donor's surgery. The isolated endothelial cells are then deposited on a graft for implantation.

#### Summary of the Invention

10 A device for collecting and processing tissue to produce an endothelial cell product is provided and includes a vessel for rinsing, draining, digesting and isolating tissue. The vessel has a rinsing and digesting chamber for containing tissue during processing. An inlet is in the rinsing and  
15 digesting chamber for connection in fluid communication with a liposuction means. A drain chamber or waste chamber is preferably provided in the vessel and is in fluid communication with the rinsing and digesting chamber. The waste chamber has a connection for fluid communication with a vacuum source. A  
20 screen is in the vessel for separating the rinsing and digesting chamber from the waste chamber to allow filtered transfer of rinsing solution from the rinsing and digesting chamber to the waste chamber.

An isolation chamber is in the vessel with the rinsing  
25 and digesting chamber and is separated from the rinsing and digesting chamber by another screen. A pair of ports are preferably separated by a channel that forms an ampule, each of which may be selectively attached in fluid communication with the ampule by a valve means. Rinsed, digested, and processed  
30 tissue, produced under sterile conditions, may be collected within the ampule as endothelial cell product. The vessel is generally cylindrical, having a frusto-conical end, to house the isolation chamber.

A preferred method of collecting and processing tissue  
35 to produce an endothelial cell product has the step of providing a collecting vessel for processing tissue to produce

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an endothelial cell product, the vessel having a rinsing and digesting chamber, a waste chamber and an isolation chamber. Another step is introducing tissue to be processed into the rinsing and digesting chamber. Another step is introducing rinsing solution into the rinsing and digesting chamber. A further step may be orienting the vessel to screen the tissue to be processed in the rinsing and digesting chamber from rinsing solution passed into the waste chamber. An additional step is introducing an enzyme into the rinsing and digesting chamber. This is followed by the steps of heating the tissue to be processed and the enzyme for a time period and at a temperature sufficient to digest, while agitating the rinsing and digesting chamber to digest the tissue with the enzyme. Centrifuging the vessel transfers the cells from the rinsing and digesting chamber into the isolation chamber. As a further step, the pellet of microvessel endothelial cells is isolated by selective operation of a valve means.

#### Brief Description of the Drawings

FIG. 1 is a perspective view of a device for collecting and processing tissue to produce an endothelial cell product;

FIG. 2 is a partial sectional view of the device of FIG. 1 taken along lines 2-2 of FIG.1, and showing the valve handles in a first position during centrifugation;

FIG. 2A is a schematic of the path of the flow through the valves and ports during centrifugation;

FIG. 3 is a partial sectional view of the device of FIG. 1 similar to that of FIG. 2, but with the valve handles in a second position after centrifugation;

FIG. 3A is a schematic of the possible paths of the flow through the valves and ports after centrifugation;

FIG. 4 is a side elevational view, partially in section, showing a device similar to that of FIGS. 1-3, but with a modified filter arrangement illustrating the invention;

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FIG. 5 is a side elevational view, partially in section, showing a modification of the device of FIG. 4 and further illustrating the invention;

FIG. 6 is a flow diagram illustrating the steps in the process of collecting and processing tissue to produce an endothelial cell product;

FIG. 7 is a schematic of an embodiment of apparatus useful with the present invention;

FIG. 8 is an enlarged longitudinal cross-section of another embodiment of a collection and processing device;

FIG. 9 is an enlarged longitudinal cross-section of another embodiment of the collection and processing device.

#### Detailed Description of the Invention

In accordance with the preferred methods useful with the present invention, subcutaneous fat is removed from a patient using modified liposuction techniques and transferred to a self-contained, closed device where the fat can be stored under sterile conditions until needed. The fat is sterilely transferred to a digestion device where it is initially washed to remove red blood cells and other debris, followed by a controlled collagenase digestion for about 20 minutes at about 37 C. The fat slurry is then transferred to an endothelial cell isolation device, again under sterile conditions, where endothelial cells sediment into an isolation device, allowing automatic retrieval of the isolated endothelial cells. The cell suspension is then sterilely transferred to a processing unit wherein the cells are rapidly filtered onto the graft surface under sterile conditions. The endothelial cell isolation and deposition process requires only about 40 minutes for completion using the apparatus described herein. Following an incubation period, the graft is ready for implantation into the patient. In paired comparisons between the present invention and the methods practiced previously, equivalence and reproducibility in the number of isolated endothelial cells and adherence of the cells to graft surface have been observed. The system yields endothelial cell product in numbers

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acceptable for subsequent high density seeding, e.g., in a range of about  $5.14 \times 10^6$  to  $4.24 \times 10^7$  cells from 50 cc of fat, and adherence to the graft surface. The apparatus of the present invention deposits cells along the entire length and diameter of the graft consistently, with no significant difference in cell concentration as compared by analysis of variance. Significant advantages of the technique to which the present invention is preferably applied are: 1) closed, sterile fluid path; 2) minimal user input; 3) compatibility with an operating room environment; and 4) optimization of the conditions to a highly reproducible process from patient to patient.

Generally, a system wherein the present invention is useful comprises of five primary subsystems: 1) fat collection unit; 2) digestion unit; 3) endothelial cell isolation unit; 4) vascular graft processing unit; endothelial cell deposition unit. The fat collection unit collects subcutaneous fat tissue sample from a patient. A schematic of a fat collection unit which is used to collect fat containing microvascular endothelial cells from a patient to receive a graft, which fat is ultimately collected into a fat collection device, is illustrated in FIG. 7. The components include: in-flow tubing 112, fat collection device 114, vacuum tubing 115, aspiration cannula 110 and an aspiration pump 118. The aspiration pump 118 is used to suction subcutaneous fat tissue from the patient through the cannula 110 and in-flow tubing 112 and into the fat collection device 114.

Referring now to FIG. 1 there is shown generally an embodiment of the device 10 of the present invention for collecting and processing tissue to produce an endothelial cell product. In accordance with certain aspects of the present invention, the fat collection and processing functions described above are performed in a single unit as shown. A vessel 11 is used for rinsing, draining, digesting and isolating tissue for the device 10. As shown in the side elevational views of FIGS. 2-5, the vessel 11 is comprised of two parts 12,13. While not required, the vessel 11 is most

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preferably made of a low cost polymer that is easy to shape and resistant to the substances that will be handled during processing endothelial cells. In a preferred embodiment the upper part 12 is an inverted cup 14, and the lower part 13 is a frusto-conical funnel 15 with a top edge 16 that conjugates with an open bottom 17 of the inverted cup 14 to form an enclosure 18 in the vessel 11. A rinsing and digesting chamber 19 is defined within this enclosure 18. The rinsing and digesting chamber 19 is contained within the inverted cup 14 of the vessel 11 so that tissue can be suitably handled during processing. An inlet 20 is provided in side 21 of the rinsing and digesting chamber 19 for connection in fluid communication with an external liposuction means such as the cannula 110 shown in FIG. 7, for introducing tissue having microvessel endothelial cells into the rinsing and digesting chamber 19.

As shown in FIG. 2, a drain chamber or waste chamber 22 is located within an upper inside portion 23 of the inverted cup 14 of the vessel 11 and is in fluid communication with the rinsing and digesting chamber 19. In FIG. 4, an alternate drain chamber or waste chamber 24 is illustrated that is centrally located within enclosure 18. Both waste chambers 22, 24 have a connection 25 located to pass through a top 26 of the inverted cup 14 for allowing fluid communication with a vacuum source. In FIG. 5, a device similar to that of FIGS. 2-4 is shown, but the connections of 20 and 25 are reversed. Specifically, the inlet in FIG. 5 is 25' and the outlet connection is 20'; the waste chamber is thus now 19' and the rinsing and digesting chamber is 24'. The rinsing and digesting chamber 24' is centrally located within enclosure 18.

In FIG. 2, an upper screen 27 positioned within the vessel 11, inside inverted cup 14, separates the rinsing and digesting chamber 19 from the waste chamber 22 to allow filtered transfer of rinsing solution from the rinsing and digesting chamber 19 to the waste chamber 22. The screen 27 may be of various configurations and as shown in FIG. 2, the screen 27 is preferably flat. As a result, the waste chamber 22 is adjacent to upper inside portion 23 of the inverted cup

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14, and the rinsing and digesting chamber 19 is immediately beneath. In FIG. 4 the screen 28 is shown in the shape of a cylinder, which defines the waste chamber 24 within the center of the inverted cup 14 along an axis "A" thereof. The rinsing and digesting chamber 19 circumscribes or fits about the screened in waste chamber 24 in a surrounding fashion.

Lower part 13 defines a frusto-conical funnel 15 with an internal chamber 29 and having a top edge 16 which is connected to the open bottom 17 of inverted cup 14 to form a combined enclosure 18,29. Referring to the embodiments of FIGS 2-4, each preferably has a flat transverse screen 30 that is positioned adjacent top edge 16 of funnel 15. It should be noted that the embodiment of FIG. 5 does not include such a screen 30. A tube 31 connects to the apex 32 of the funnel 15. The tube 31 carries a pair of spaced apart ports 34,35, connected to a vertical channel that forms an ampule 36, positioned centrally within the tube 31. Ports 34,35 are for selective communication with the ampule 36 that is accomplished by valves 37,45, respectively, as shown in FIG. 4. The valves 37,45 may be simple stopcocks as are commonly found in medical accessories. To achieve this, valve 37 is rotated as indicated by arrow 46, and valve 45 is rotated as indicated by arrow 47. Arrows 48, 49 show flow direction two ways through ports 34,35. Using valves 37,45 permits rinsed, digested and processed tissue produced under sterile conditions to be isolated within the ampule 36 as a microvessel endothelial cell pellet. To remove the pellet, the valves 37,45 are adjusted to allow the pellet to be drawn off through one of ports 34,35.

FIG. 2A shows a schematic view of the flow path through the valves 37,45 during centrifugation. This flow path traps red blood cells in a trap 38 at the bottom of ampule 36, as shown in FIG. 2A. The trap 38 collects red blood cells during centrifugation because they are denser than the microvessel endothelial cells. After centrifugation, the valves 37, 45 can be adjusted as shown schematically in FIG. 3A. As shown, two flow paths are possible such that the

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isolated cells can be removed from ampule 36 and trap 38 by vacuum or pressure applied to either ports 34,35.

In another aspect of the present invention, a method of collecting and processing tissue to produce an endothelial  
5 cell product is also disclosed. The various steps in a preferred embodiment of this process are shown on the schematic diagram of FIG. 6. To perform the method, a vessel 11 for processing tissue to produce an endothelial cell product such as that described herein is provided. The vessel 11 preferably  
10 has a rinsing and digesting chamber 19, a waste chamber 22,24 and an isolation chamber 29.

Introducing tissue to be processed into the rinsing and digesting chamber 14 is a preliminary step of the processing method. The tissue may be obtained from a  
15 liposuction procedure. Orienting the vessel permits vacuum and gravity to filter off unneeded liquid from the tissue sample through a screen 30, which separates the tissue to be processed in the rinsing and digesting chamber 19 from rinsing solution which passes into the waste chamber 22,24. The method is  
20 continued by introducing an enzyme into the rinsing and digesting chamber 19, and heating the tissue to be processed in the presence of an enzyme for a period of time, and at a temperature sufficient to digest the tissue. The digestion is assisted by the step of agitating the rinsing and digesting  
25 chamber 19 containing the tissue and enzyme.

Centrifuging the vessel about the top 26 of the inverted cup 14 collects the cells from the digested tissue from the rinsing and digesting chamber 19 in the isolation chamber 29. Any red blood cells in the rinsed and digested  
30 tissue will be collected in the trap 38 at the bottom of ampule 36 because, as mentioned previously the red blood cells are denser and heavier than the microvessel endothelial cells. The method also preferably includes the further step of isolating the digested tissue in the form of a pellet of microvessel  
35 endothelial cells by selective application of the valves 37,45, as discussed above.

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In accordance with the present invention, the fat collection and processing functions can also be performed in other embodiments of a single device, as shown in FIG. 8. As true for the embodiments shown in FIGS. 1-5, this embodiment is  
5 designed as a process vessel to collect, rinse and digest fat tissue and then separate and isolate microvessel endothelial cells for research, diagnostic or therapeutic purposes, e.g., endothelialization of a prosthetic or natural surface. The device has three chambers: a digestion chamber 19, a waste  
10 chamber 22, and an isolation chamber 29. The digestion chamber 19 is separated from the waste chamber 22 by a plate 218 containing a normally closed check valve 220. A vent tube 222, containing a floating ball check valve 224 extends from the waste chamber 22 into the isolation chamber 29. The digestion  
15 chamber 19 communicates with the outside by means of a series of ports 20,229,230. The digestion chamber 19 is separated from the isolation chamber 29 by a screen 30. The isolation chamber 29 possesses two ports 34,35, each of which contains a valve 37,45. The first position of each valve 37,45 allows  
20 communication between the middle of an ampule 36 formed between them. The second position of the valves 37,45 allows communication between the ampule 36 and the outside ports 34,35. When practicing the methods disclosed herein, it is preferred that both ampule valves 37,45 are initially in a  
25 first position, and the device shown in FIG. 8 is used as a catch-trap in line with a liposuction vacuum line connected to two of the ports 20,230 that connect to the digestion chamber 19. After fat is collected, the liposuction lines are disconnected and the ports 20,230 are capped. Rinse solution  
30 (e.g., Media 199E, Hanks, saline, PBS, or other physiological buffered solution) is introduced through another port 229. The fat is agitated in the rinse solution by any external means such as shaking on a shaker table, manual agitation, etc. The device is then placed in a centrifuge, ampule side up, and spun  
35 until the normally closed check valve 220 opens and the rinse solution wastes into the waste chamber 22. The ball valve 224 in the vent tube 222 opens during this centrifugation step

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allowing the waste chamber 22 to vent any air which is displaced by the rinse solution. Digestive enzyme solution such as collagenase, dispase, trypsin, or other tissue dissociation enzyme is then introduced through the same port 5 229 as the rinse solution, again followed by agitation. When digestion is complete, the device is again centrifuged, ampule side down. In order to isolate the endothelial cells which have separated into the ampule 36, both valves 37,45 are turned to their second positions. An endothelial cell "pellet" is 10 then flushed out by attaching a pressure line to one of the ampule ports 234, 236.

In another embodiment shown in FIG. 9 a device is provided that comprises a vessel with four chambers: a digestion chamber 19, a waste chamber 22, an enzyme chamber 15 213, and an isolation chamber 29. The digestion chamber 19 is separated from the waste chamber 22 by a plate 218 containing a normally closed check valve 220. A screen 216 covers the inlet of the check valve 220. A vent tube 222, containing a floating ball check valve 224, extends from the waste chamber 20 22 into the isolation chamber 29. The waste chamber is separated from the enzyme chamber by a plate 217. The digestion chamber 19 is separated from the enzyme chamber 213 by a plate 218 containing a normally closed check valve 221. A vent tube 223, containing a floating ball check valve 225 25 extends from the digestion chamber 19 into the top portion of the enzyme chamber 213. The enzyme chamber 213 communicates with the outside by means of a port 226. The digestion chamber 19 communicates with the outside by means of a series of ports 20,229,230. The digestion chamber 19 is separated from the 30 isolation chamber 29 by a screen 30. The isolation chamber 29 possesses two ports 34,35, each of which contains a valve 37,45 that in their first position allow communication between the middle of the ampule 36 and the upper and lower portions of the ampule 36. The second position allows communication between 35 the middle of the ampule 36 and the outside ports 34,35.

Initially, both valves 37,45 are in the first position. The device is used as a catch-trap in line with a

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liposuction line connected to two of the ports 20,230. After fat is collected, the liposuction lines are disconnected and ports 228 and 230 are capped. Rinse solution (e.g., Media 199E, Hanks, saline, PBS, or other physiological buffered solution) is introduced through port 229 and digestive enzyme solution (e.g., collagenase, dispase, trypsin, or other tissue dissociation enzyme) is introduced through port 226. The fat is agitated in the rinse solution by an external means such as shaking. The device is then placed in a centrifuge, ampule side up, and spun until the normally closed check valve 220 opens and the rinse solution drains into the waste chamber 22. The ball valve 224 in the vent tube 222 opens during this centrifugation step, allowing the waste chamber 22 to vent any air displaced by the rinse solution. The device is then placed in a centrifuge, ampule side down, and spun until the normally closed check valve 221 opens and enzyme solution drains into the digestion chamber 19. The ball valve 225 in the vent tube 223 opens during this centrifugation step allowing the digestion chamber 19 to vent air which is displaced by enzyme solution. The fat is then agitated in the enzyme solution to promote digestion. When digestion is complete, the device is again centrifuged, ampule side down. In order to isolate the endothelial cells which have separated into the ampule 36 both valves 37,45 are turned to their second positions. The endothelial cell pellet may then be flushed out by attaching a pressure line to one of the ampule ports 234,236.

While the forms of apparatus and the method described herein constitute preferred embodiments of the invention, it is to be understood that the invention is not limited to these preferred forms of apparatus and methods, and that changes can be made therein without departing from the inventive concepts disclosed herein. For example, various screen pore configurations may be developed to enhance separation. Moreover, centrifugal force control and agitation intensity may be varied to enhance output quantity and quality of the final cell pellet. In addition, the main digestion chamber, while shown in cylindrical form in cross-section, may be configured

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to be square for certain operating room accommodations. Therefore, reference should be made to the appended claims in order to ascertain the true scope of the present invention.

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What is claimed is:

1. A device for collecting and processing tissue to produce an endothelial cell product comprising:  
a vessel having a rinsing and digesting chamber, a drain  
5 chamber, and an isolation chamber which chambers allow the rinsing, digestion and processing of tissue to produce within the vessel under sterile conditions endothelial cell product.
2. The device of claim 1 wherein the rinsing and digesting chamber is separated from the waste chamber by a  
10 screen.
3. The device of claim 2 wherein the rinsing and digesting chamber is separated from the isolation chamber by a screen.
4. The device of Claim 2 wherein said screen  
15 separates said rinsing and digesting chamber from said waste chamber and said isolation chamber, said waste chamber surrounds said rinsing and digesting chamber; and said waste chamber has a connection to a vacuum source.
5. The device of Claim 4 wherein the rinsing and  
20 digesting chamber includes an inlet for connection in fluid communication with a source of fat.
6. The device of claim 1 wherein the isolation chamber comprises a pair of ports connected by a channel, thereby forming an ampule; and valve means for selective fluid  
25 communication between the ampule and each of the pair of ports to control flow to and from said ampule through each of the ports.
7. A device for collecting and processing tissue to produce an endothelial cell product, comprising a vessel for  
30 rinsing, draining, digesting and isolating tissue comprising:

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a rinsing and digesting chamber in the vessel for collecting and processing tissue;

an inlet in the rinsing and digesting chamber for fluid communication with rinsing solution and a liposuction  
5 means;

a waste chamber in fluid communication with the rinsing and digesting chamber, the waste chamber having a connection for fluid communication with a vacuum source;

a first screen separating the rinsing and digesting  
10 chamber from the waste chamber to allow filtered transfer of rinsing solution from the rinsing and digesting chamber to the waste chamber;

an isolation chamber in fluid communication with and separated from the rinsing and digesting chamber by a second  
15 screen to allow cells from rinsed, digested and processed tissue produced under sterile conditions to be isolated;

an ampule in fluid communication with said isolation chamber;

a pair of ports in fluid communication with the  
20 ampule; and

valve means operatively associated with each of the ports to selectively control fluid communication between each of the ports, the ampule and the isolation chamber,

endothelial cells disposed in the ampule are isolated  
25 by selective operation of the valve means.

8. A device for digesting and processing tissue to produce an endothelial cell product comprising:

a single vessel comprising three chambers: a digestion chamber; a separate waste chamber; and a separate  
30 isolation chamber selectively in fluid communication with the waste chamber, wherein the isolation chamber is selectively connected by a valve to an ampule for receiving said endothelial cell product, and wherein the digestion chamber is selectively and alternately in fluid communication with the  
35 waste chamber and the isolation chamber,

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whereby the device allows the digestion of tissue by connecting the digestion chamber to the waste chamber and processing of tissue by connecting the digestion chamber to the isolation chamber to produce endothelial cell product within a  
5 single vessel under sterile conditions.

9. The device of claim 8 further comprising a normally closed check valve connecting the digestion chamber and the waste chamber.

10. The device of claim 9 further comprising an  
10 internal vent tube for permitting flow through the check valve, the vent tube extending from the waste chamber into the isolation chamber.

11. The device of claim 8 further comprising a first and a second valve, each valve having at least two positions,  
15 wherein in a first position the first valve allows communication between the digestion chamber and the ampule, and a second valve allows communication between the ampule and an outlet port, and wherein, in a second position the valves isolate endothelial cell product within the ampule.

20 12. The device of claim 8 further comprising at least one inlet port affixed to the vessel in communication with the digestion chamber for introducing collected fat, rinse and enzyme solutions into said digestion chamber.

13. A device for digesting and processing tissue to  
25 produce an endothelial cell product comprising:

a single vessel comprising four chambers: (a) an enzyme storage chamber; (b) a separate waste chamber; (c) a separate digestion chamber alternately and selectively connected to the waste chamber and the enzyme chamber; and (d)  
30 a separate isolation chamber selectively connected to the digestion chamber, wherein the isolation chamber is selectively connected by a first valve to an ampule for receiving said

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endothelial cell product, whereby the device allows the digestion of tissue by connecting the digestion chamber to the enzyme storage chamber and the waste chamber and processing of tissue by connecting the digestion chamber to the isolation  
5 chamber to produce endothelial cell product within a single vessel under sterile conditions.

14. The device of claim 13 wherein said enzyme storage chamber selectively communicates with said digestion chamber through a valve and said enzyme storage chamber  
10 communicates with an external environment through a port.

15. The device of claim 14 wherein the valve between said enzyme storage chamber and said digestion chamber is a normally closed check valve which opens when pressure increases inside said enzyme storage chamber upon centrifugation of said  
15 device.

16. The device of claim 13 wherein said digestion chamber further comprises a means to introduce tissue to be digested within said digestion chamber, and a means to introduce rinsing fluid into said digestion chamber for rinsing  
20 said tissue.

17. Apparatus for digesting and isolating tissue to produce an endothelial cell product comprising:

a sealed digestion chamber comprising a chamber for holding tissue that includes a first inlet port for admitting tissue; a second inlet port for admitting enzymes; a third inlet port for admitting  
25 rinse solution; a fourth inlet port to admit gas to the sealed digestion device to agitate the tissue; and an outlet port for selectively expelling digested tissue and retaining undigested tissue; and  
30

an isolation chamber selectively connected to the digestion device and also selectively connected

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by a valve to an ampule for isolating the endothelial cell product.

18. A method for collecting and processing tissue for producing an endothelial cell product comprising:

- 5           providing a collecting vessel for processing tissue to produce an endothelial cell product, the vessel having a rinsing and digesting chamber in fluid communication with a separate waste chamber and an isolation chamber;
- introducing tissue to be processed into the rinsing  
10 and digesting chamber;
- introducing rinsing solution into the rinsing and digesting chamber;
- orienting the vessel to screen the tissue to be processed of rinsing solution passed into the waste chamber;
- 15           introducing an enzyme into the rinsing and digesting chamber;
- heating the tissue and the enzyme for a sufficient time and temperature while agitating the rinsing and digesting chamber to digest the tissue with the enzyme;
- 20           centrifuging the vessel to transfer cells from the digested tissue from the rinsing and digesting chamber into the isolation chamber; and
- isolating the cells as a pellet of microvessel endothelial cells.

- 25           19. The method of claim 18 with the further step of isolating the cells from the digested tissue in the ampule as a pellet of microvessel endothelial cells by application of a valve means.

## AMENDED CLAIMS

[received by the International Bureau on 21 December 1993 (21.12.93); original claims 2-5 and 9 cancelled; original claims 1,6-8,10,11,13 and 17 amended; new claims 2-5 and 9 added; other claims unchanged (9 pages)]

1. In a device for collecting and processing tissue to produce an endothelial cell product, the improvement comprising providing a single vessel comprising:
  - a rinsing and digesting chamber for receiving tissue;
  - a separate waste chamber surrounding the rinsing and digesting chamber, the waste chamber for receiving waste fluid from the rinsing and digesting chamber after the waste fluid has flowed through a first screen;
  - a separate isolation chamber for receiving endothelial cell product from the rinsing and digesting chamber, wherein the first screen comprises single screen structure that separates the rinsing and digesting chamber from the waste chamber and the isolation chamber, and the rinsing and digesting chamber is separated from the isolation chamber by a second screen;
  - a connection between the waste chamber and a vacuum source;
  - an inlet port connecting the rinsing and digesting chamber to a source of fat; and
  - an outlet port in the waste chamber for selectively withdrawing the waste fluid,wherein the waste and isolation chambers allow the rinsing, digestion and processing of tissue to produce endothelial cell product within the vessel under sterile conditions.

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6. The device of claim 1 wherein the isolation chamber is selectively connected to the isolation chamber by an outlet using a pair of ports connected by a channel, thereby forming an ampule; and valve means for selective fluid communication between the ampule and each of the pair of ports to control flow to and from the ampule through each of the ports.

7. In a device for collecting and processing tissue to produce an endothelial cell product, the improvement comprising a single vessel for rinsing, draining, digesting and isolating tissue, the vessel comprising:

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a rinsing and digesting chamber in the vessel for collecting and processing tissue;

an inlet connected to the rinsing and digesting chamber for receiving rinsing solution and also for receiving tissue from a liposuction means for withdrawing tissue from a subject;

a waste chamber in fluid communication with the rinsing and digesting chamber, the waste chamber further comprising a connection for fluid communication with a vacuum source;

a first screen separating the rinsing and digesting chamber from the waste chamber wherein waste fluid comprising rinsing solution flows from the rinsing and digesting chamber through the first screen to the waste chamber;

an isolation chamber in fluid communication with and separated from the rinsing and digesting chamber by a second screen to allow cells from rinsed, digested and processed tissue produced under sterile conditions to be isolated;

an ampule connected to and in selective fluid communication with the isolation chamber;

a pair of ports providing a selective connection between the isolation chamber and the ampule; and

valve means operatively associated with each of the ports to selectively control fluid communication between each of the ports, the ampule and the isolation chamber,

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whereby endothelial cells disposed in the ampule are isolated by selective operation of the valve means.

8. In a device for digesting and processing tissue to produce an endothelial cell product the improvement comprising providing a single vessel comprising three chambers:

a digestion chamber; a separate waste chamber; and a separate isolation chamber, the waste and isolation chambers in fluid communication with the digestion chamber, wherein the isolation chamber is selectively connected by a valve to an ampule for receiving the endothelial cell product, and wherein the digestion chamber is selectively and alternately placed in fluid communication with the waste chamber and the isolation chamber, by the operation of a normally closed check valve connecting the digestion chamber and the waste chamber,

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whereby the device allows the digestion of tissue by connecting the digestion chamber to the waste chamber, and processing of tissue by connecting the digestion chamber to the isolation chamber to produce endothelial cell product within the single vessel under sterile conditions.

10. The device of claim 8 further comprising an internal vent tube for permitting flow through the check valve, the vent tube extending from the waste chamber into the isolation chamber and a valve regulating fluid flow between the waste chamber and the isolation chamber.

11. The device of claim 8 further comprising a first and a second valve, each valve having at least two positions, wherein in a first position the first valve allows communication between the isolation chamber and the ampule, and a second valve allows communication between the ampule and an outlet port, and wherein, in a second position the valves isolate endothelial cell product within the ampule.

12. The device of claim 8 further comprising at least one inlet port affixed to the vessel in communication with the digestion chamber for introducing collected fat, rinse and enzyme solutions into said digestion chamber.

13. In a device for digesting and processing tissue to produce an endothelial cell product the improvement comprising providing a single vessel comprising:

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four chambers: (a) an enzyme storage chamber;  
(b) a separate waste chamber; (c) a separate digestion  
chamber alternately and selectively connected to the waste  
5 chamber and the enzyme chamber; and (d) a separate isolation  
chamber selectively connected to the digestion chamber,  
wherein the isolation chamber is selectively connected by a  
first valve to an ampule for receiving said

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endothelial cell product, whereby the device allows the digestion of tissue by connecting the digestion chamber to the enzyme storage chamber and the waste chamber and  
5 processing of tissue by connecting the digestion chamber to the isolation chamber to produce endothelial cell product within a single vessel under sterile conditions.

14. The device of claim 13 wherein said enzyme storage chamber selectively communicates with said digestion chamber through a valve and said enzyme storage chamber communicates with an external environment through a port.

15. The device of claim 14 wherein the valve between said enzyme storage chamber and said digestion chamber is a normally closed check valve which opens when pressure increases inside said enzyme storage chamber upon  
5 centrifugation of said device.

16. The device of claim 13 wherein said digestion chamber further comprises a means to introduce tissue to be digested within said digestion chamber, and a means to introduce rinsing fluid into said digestion chamber for  
5 rinsing said tissue.

17. In an apparatus for digesting and isolating tissue to produce an endothelial cell product the improvement comprising providing a sealed digestion chamber comprising:

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a chamber for holding tissue that includes a first inlet port for admitting tissue; a second inlet port for admitting enzymes; a third inlet port for admitting rinse solution; a fourth inlet port to admit gas to the sealed digestion device to agitate the tissue; and an outlet port for selectively expelling digested tissue and retaining undigested tissue; and

an isolation chamber selectively connected to the outlet port of the digestion chamber at its first end and selectively connected

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by a valve to an ampule for isolating the endothelial cell product at its second end.

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STATEMENT UNDER ARTICLE 19(1)

Claims 2-5 and 9 have been cancelled.

The differences between the original claims and the amended claims is as follows:

The amended claims now recite the specific separate chambers used in preferred embodiments of the invention and further define the structural relationship between these chambers, including defining which chambers are separated by screens, and which chambers are selectively connected by valves or ports. In some instances, the amendments have incorporated the subject matter of dependent claims into independent claims.

The amendments set forth herein correspond substantially to amendments made in the corresponding U.S. priority application. In the priority application, certain claims have been indicated as defining subject matter neither disclosed nor suggested in the prior art of record. Thus, Applicants made the amendments set forth above to include limitations to define the invention over the prior art of record. The differences between the amended claims and the prior art is as follows:

U.S. Patent No. 2,9104,406--Novak the claimed invention, but instead discloses a series of chambers separated by screens, which is not the same as their being "selectively connected" as recited, for example, by claim 1. There is no connection, alternate, selective or otherwise between a structure that would arguably be a "digestion" chamber and two additional chambers--the "isolation" chamber and a waste chamber. Novak nowhere discloses or suggests selective and alternate connections between chambers. The disclosed chambers are simply in series.

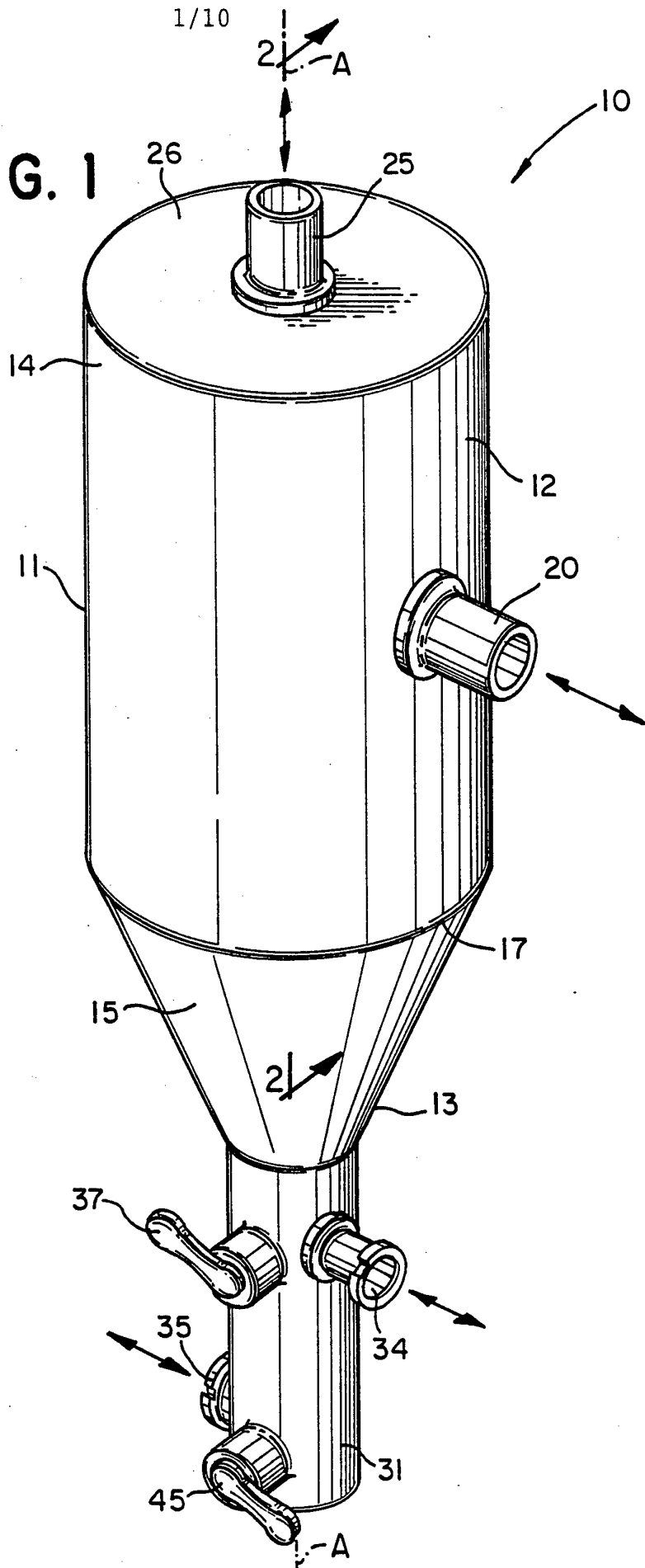
U.S. Patent No. 4,981,596--Shiino et al. discloses a blood transfusion and filtration system that uses a filter disposed within a vacuum chamber and does not disclose the port in the waste chamber that permits waste fluid to be withdrawn as recited, for example, in claim 1. The "waste chamber" of Shiino et al. is directly connected to a vacuum source that provides the suction to draw blood through the filter. Any "waste fluid" actually remains inside the filter media. Thus, Shiino et al. do not disclose or suggest the claimed invention since there is no suggestion of removing waste fluid through a port.

U.S. Patent No. 5,035,708--Alchas et al. does not disclose or suggest a single chamber having the recited chambers, nor is the recited screen disclosed. The relevant claims require a single vessel that includes the recited chambers and portions. One aspect of the claimed invention is

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the multiple ports used to selectively access the recited chambers. None of these features are disclosed or suggested by Alchas et al.

FIG. 1



SUBSTITUTE SHEET

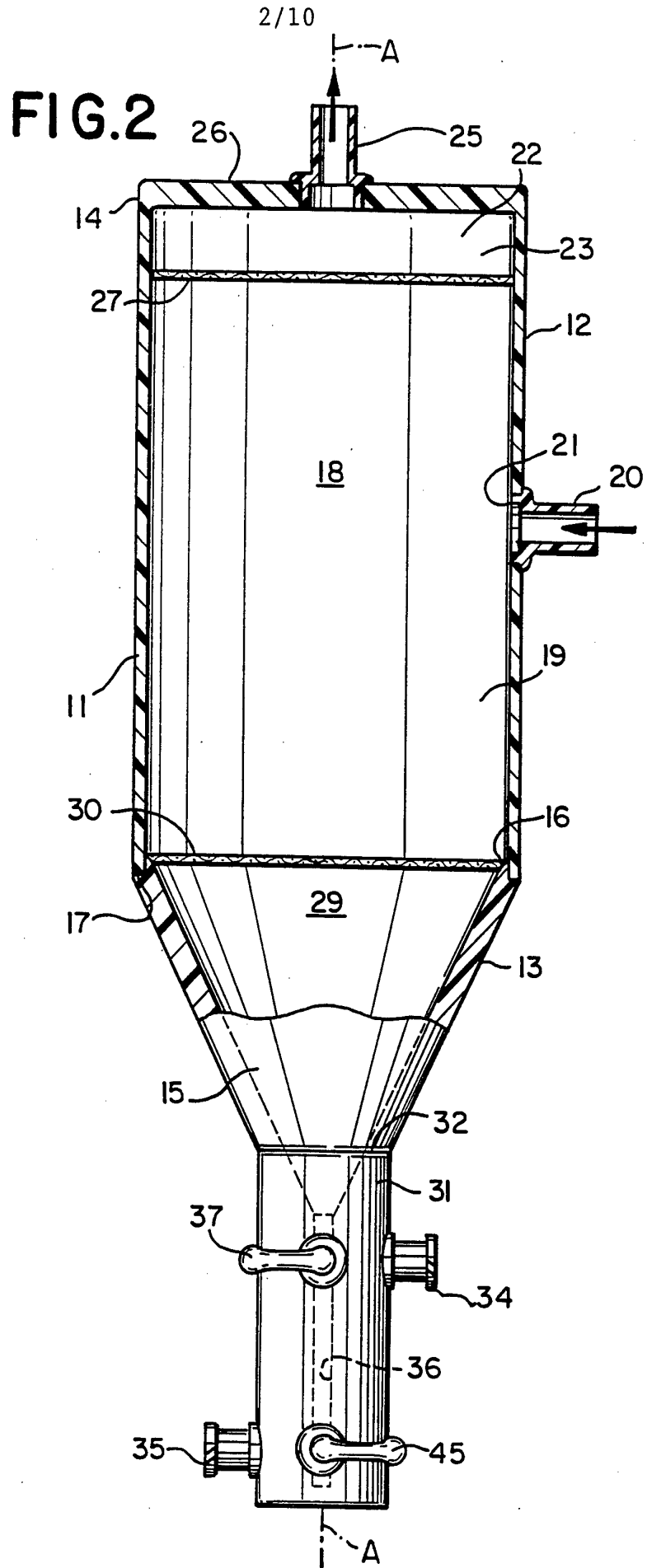


FIG. 2A

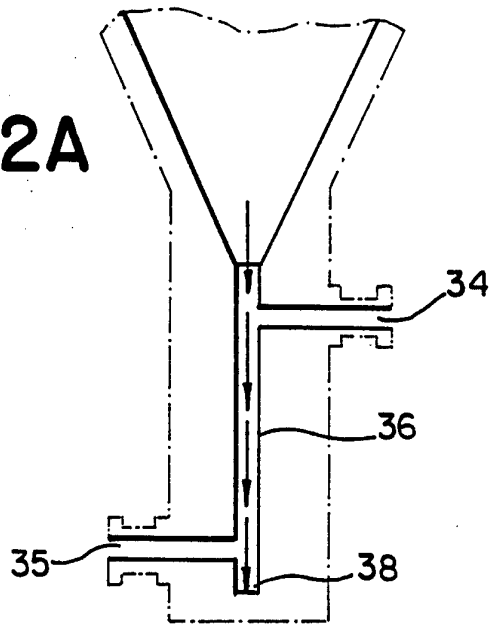


FIG. 3A

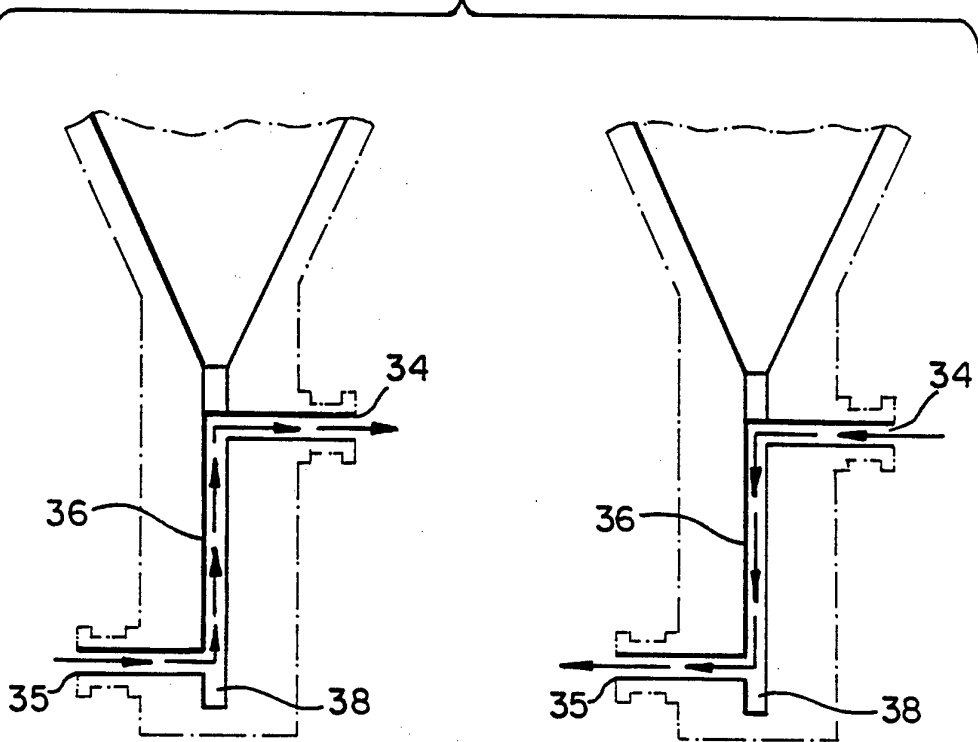
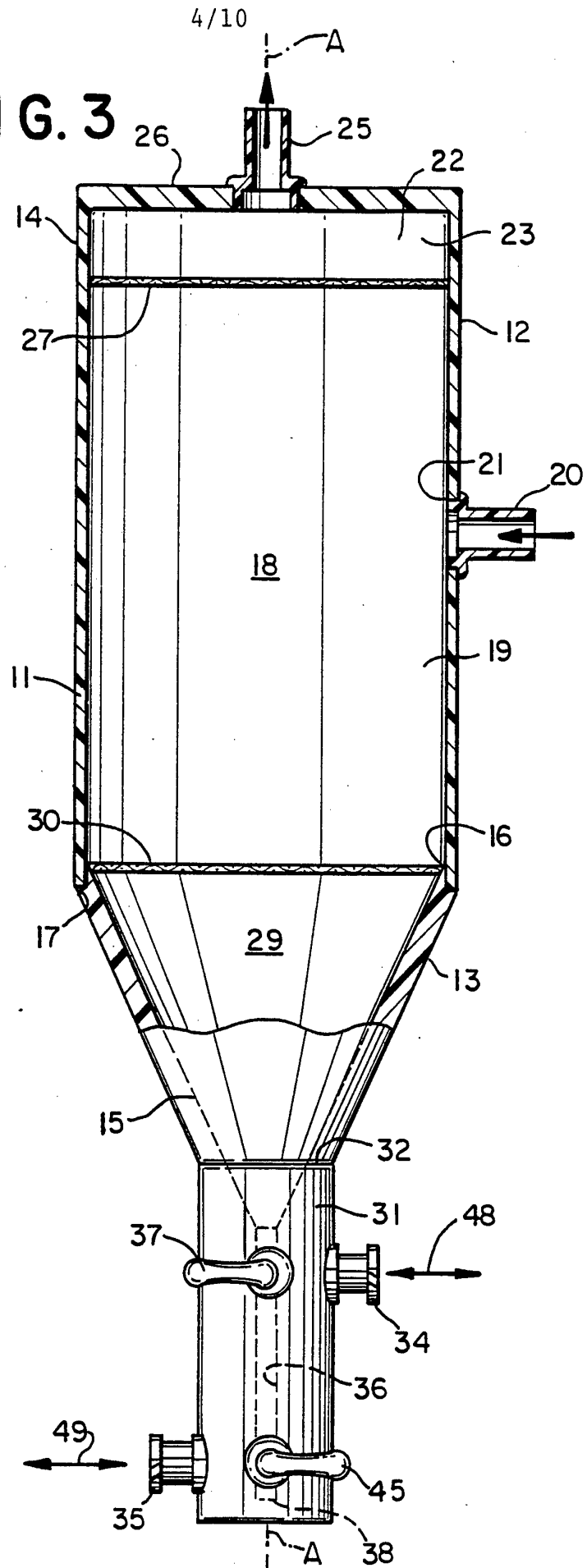


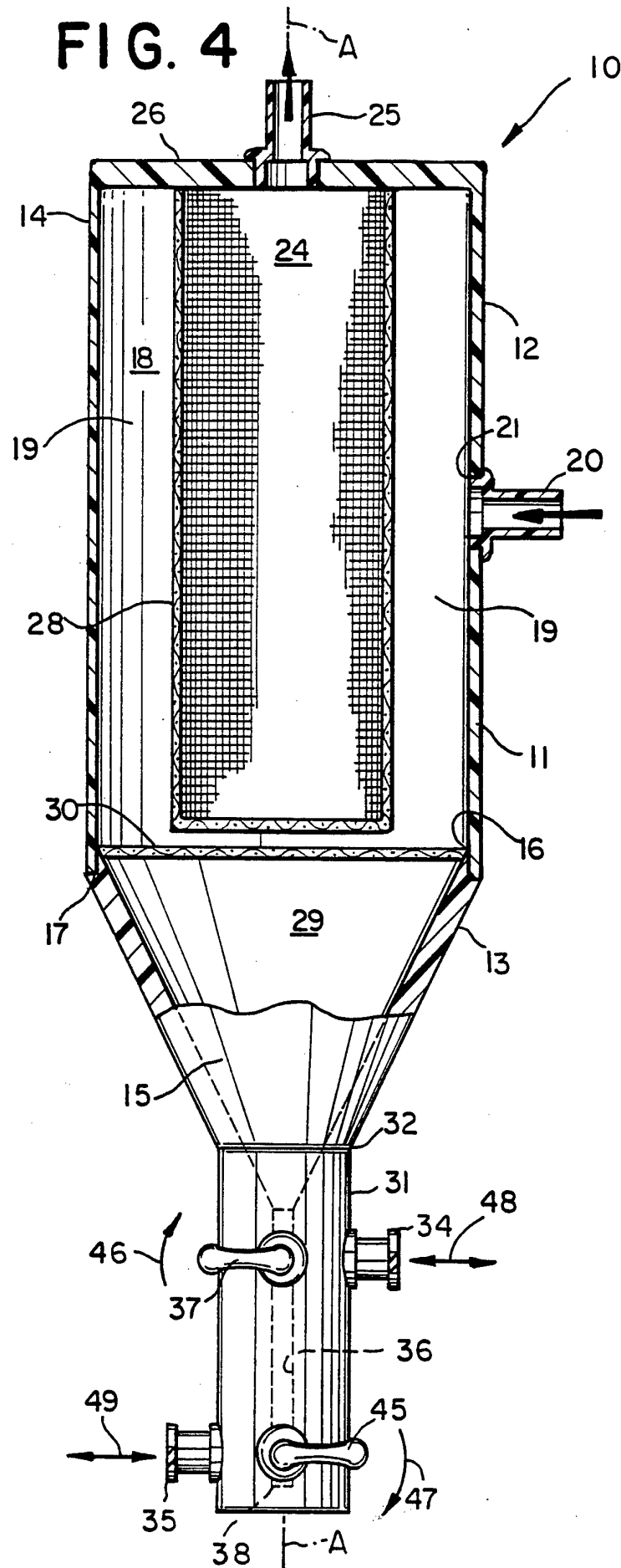
FIG. 3



SUBSTITUTE SHEET

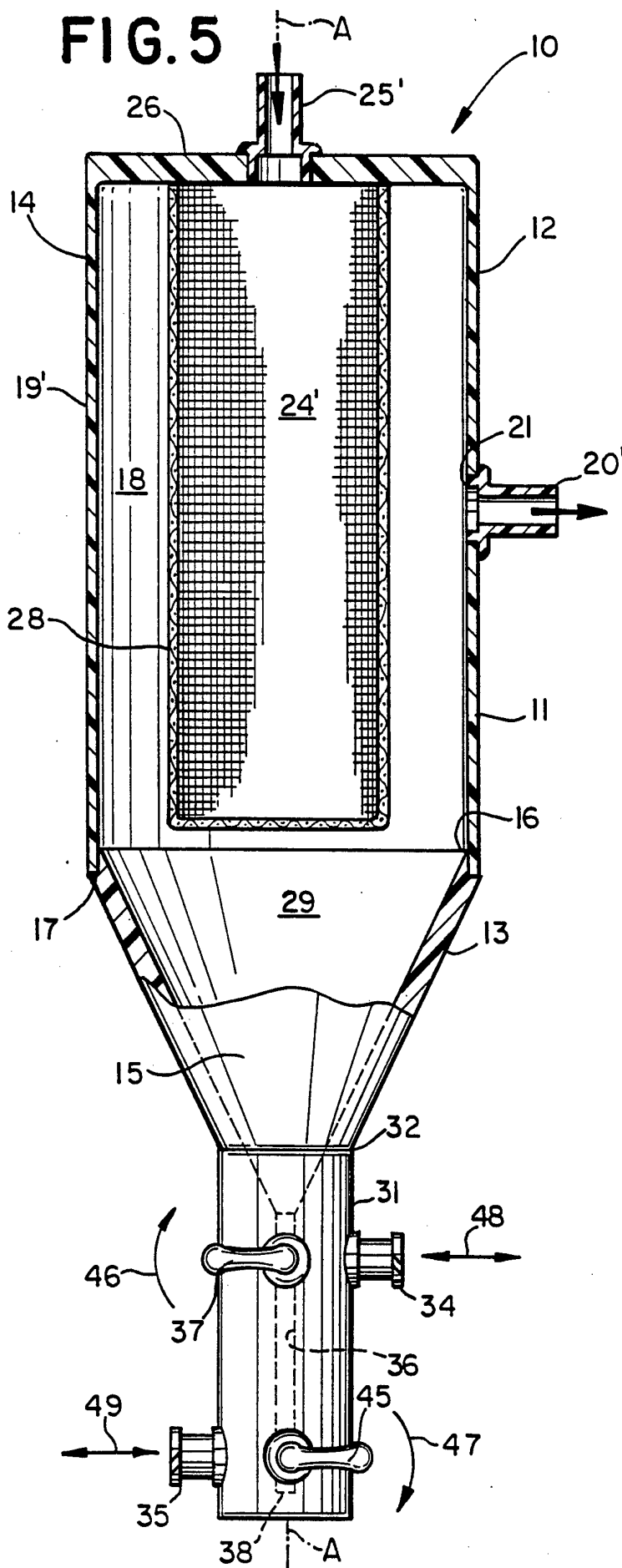
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FIG. 4

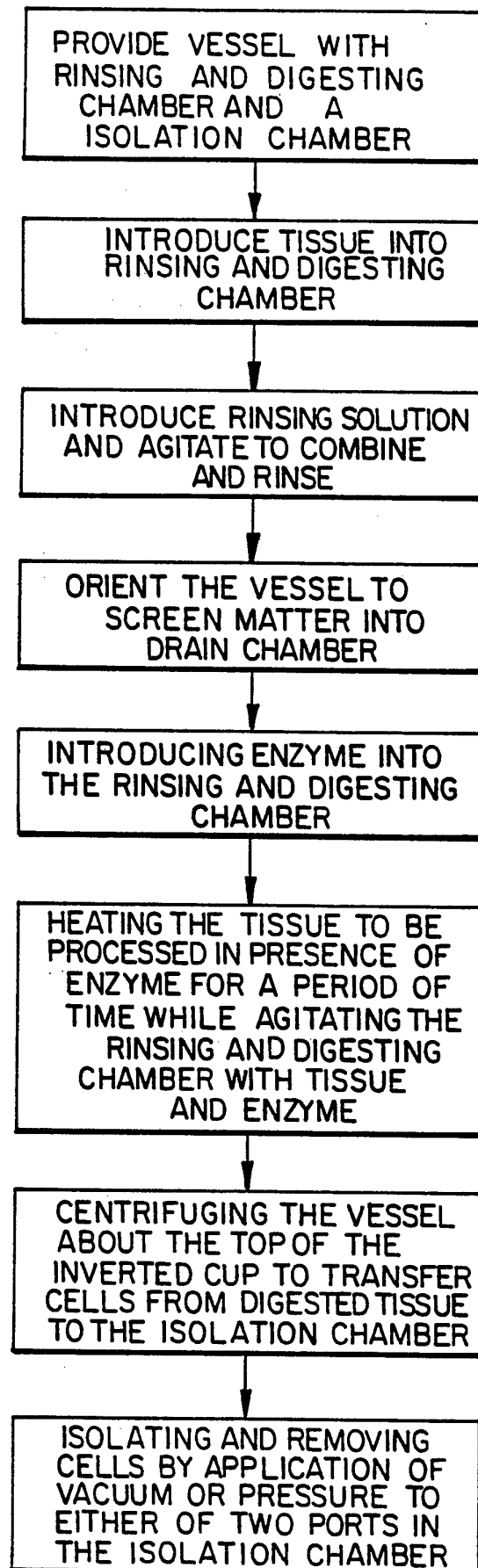


SUBSTITUTE SHEET

FIG. 5



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**FIG. 6****SUBSTITUTE SHEET**

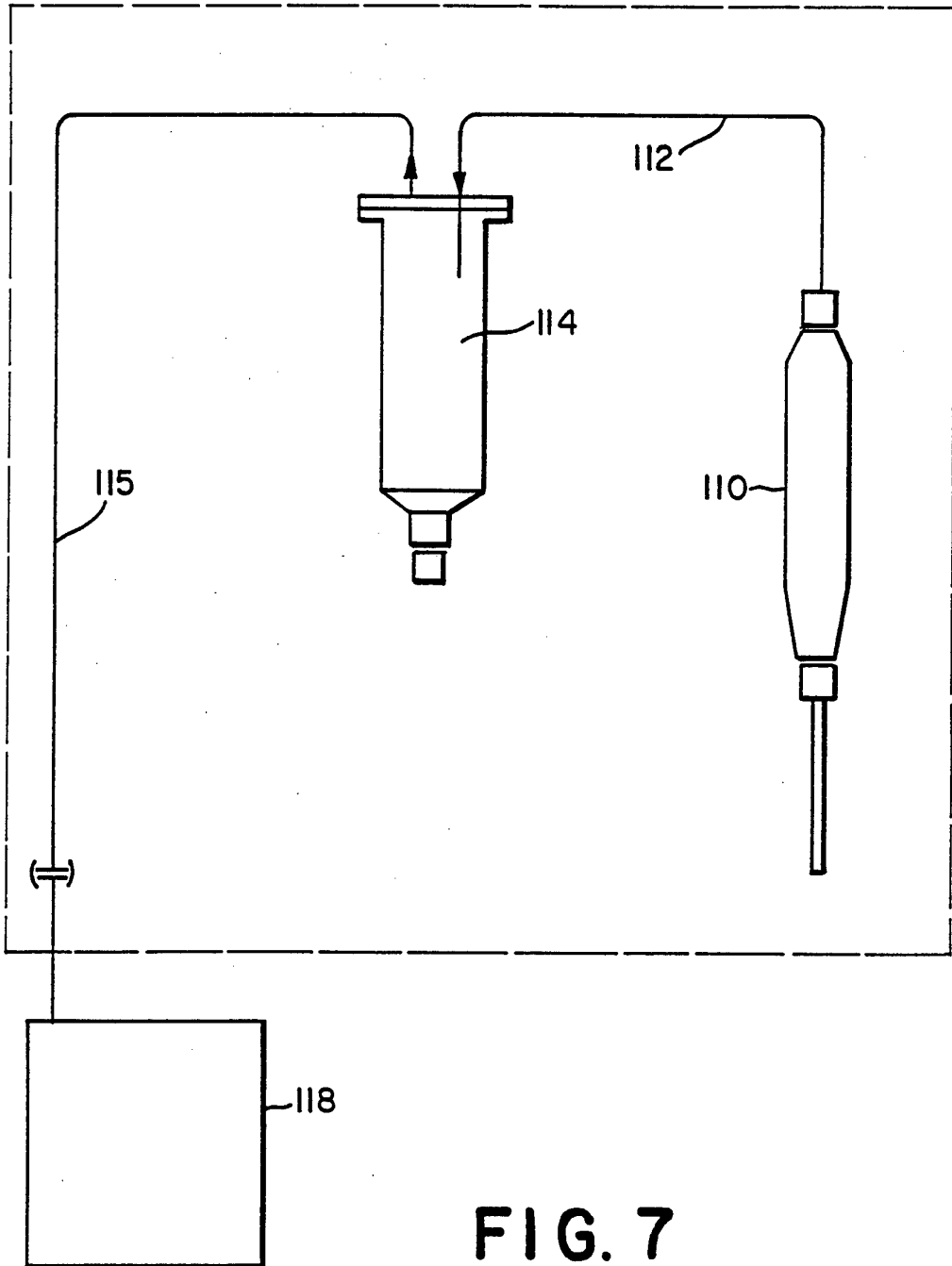
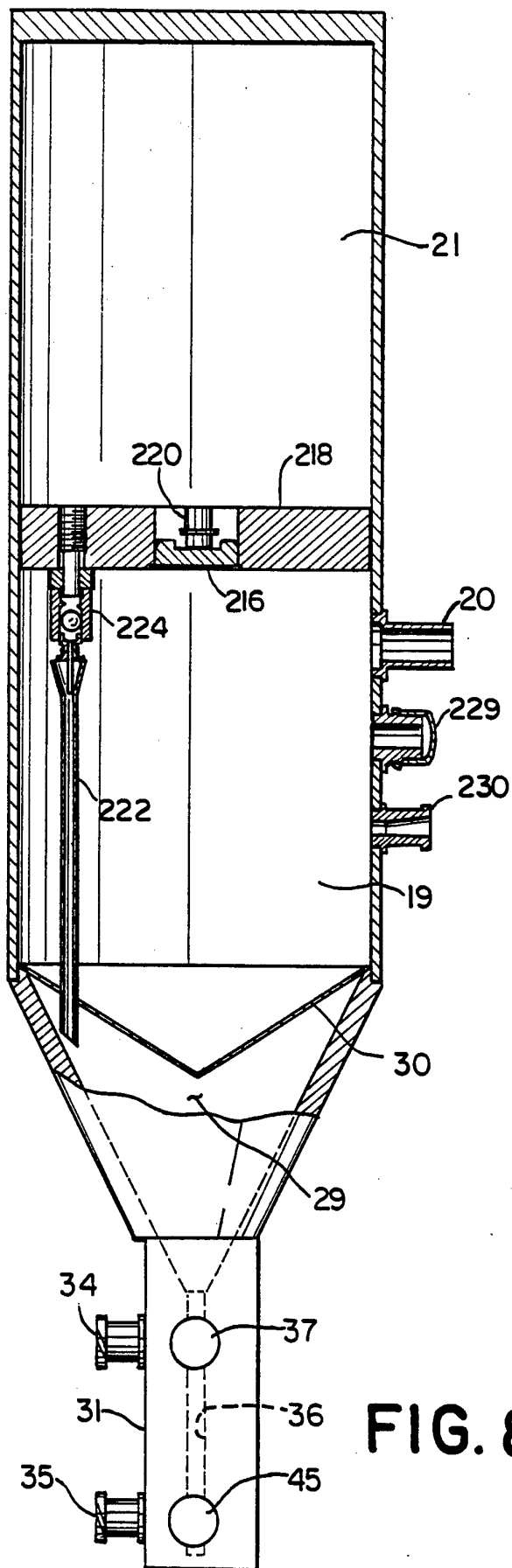
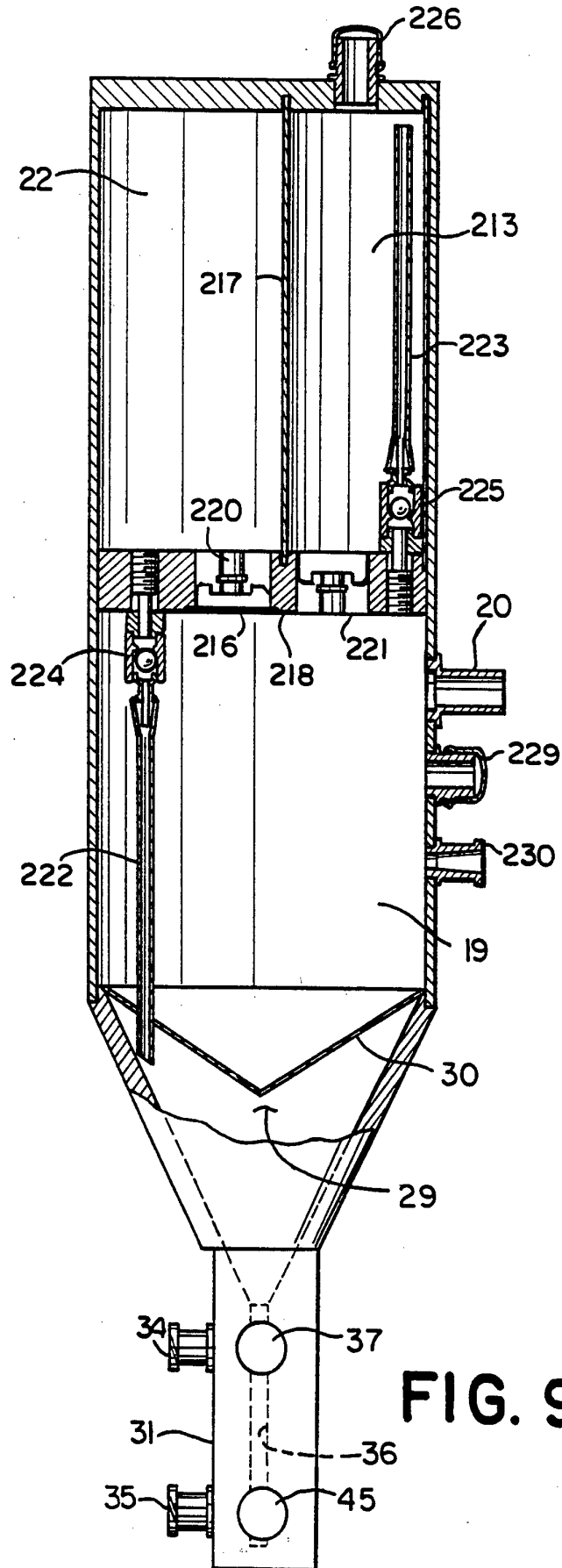


FIG. 7



**FIG. 8**




**FIG. 9**

**SUBSTITUTE SHEET**

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/07216

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(5) :C12S 3/00; C12M 1/40 US CL :435/267, 288 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : Please See Extra Sheet. 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)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 2,910,406 (NOVAK) 27 October 1959, see entire document.	1-3
X	US, A, 5,035,708 (ALCHAS ET AL.) 30 July 1991, see the entire document.	1,6
X	US, A, 4,981,596 (SHIINO ET AL.) 01 January 1991, see the entire document.	1,2,4,5
X	EP, A, 0,446,450 (ALCHAS ET AL.) 18 September 1991, see the entire document.	1-3,6-19
Y		4,5
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be part of particular relevance "E" earlier document published on or after the international filing date "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) "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 "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 "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 "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 "&" document member of the same patent family		
Date of the actual completion of the international search 13 October 1993		Date of mailing of the international search report <b>OCT 21 1993</b>
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE		Authorized officer WILLIAM H. BEISNER  Telephone No. (703) 308-0196

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US93/07216

**B. FIELDS SEARCHED**

Minimum documentation searched

Classification System: U.S.

435/1, 240.21, 262, 267, 271, 284-288, 296, 311, 316, 808; 422/72, 101-103, 113; 210/ 632, 360.1, 380.1, 406, 472;  
209/12, 19; 494/16, 27, 30, 36; 604/35, 190, 406; 128/749