637100

FORM 1

SPRUSON & FERGUSON

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

APPLICATION FOR A STANDARD PATENT

Becton Dickinson & Company, incorporated in New Jersey, of One Becton Drive, Franklin Lakes, New Jersey, 07417-1880, UNITED STATES OF AMERICA; Thomas Jefferson University, incorporated in Pennsylvania, of 617 Scott Building, 1020 Walnut Street, Philadelphia, Pennsylvania, 19107, UNITED STATES OF AMERICA, hereby apply for the grant of a standard patent for an invention entitled:

Endothelial Cell Procurement and Deposition Kit

which is described in the accompanying complete specification.

Details of basic application(s):-

Basic Applic. No:	Country:	Application Date:
356,431	US	24 May 1989

The address for service is:-

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Level 33 St Martins Tower
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DATED this TWENTY FIRST day of MAY 1990

Becton Dickinson & Company, Thomas Jefferson University

By:

Registered Patent Attorney

TO: THE COMMISSIONER OF PATENTS
OUR REF: 130678
S&F CODE: 57100

5845/2

SPRUSON & FERGUSON

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT

In support of the Convention Application made for a patent for an invention entitled:

Endothelial Cell Procurement and Deposition Kit

I, Raymond P. Ohlmuller

care of Becton Dickinson and Company, One Becton Drive, Franklin Lakes, New Jersey 07417-1880, United States of America

do solemnly and sincerely declare as follows:-

I am authorised by Becton Dickinson & Company one of the applicants for the patent to make this declaration on its behalf.

And

I, Jussi J. Saukkonen, M.D.

of 1020 Locust Street, Room M-63, Philadelphia, PA 19107 United States of America

do solemnly and sincerely declare as follows:-

I am authorised by Thomas Jefferson University one of the applicants for the patent to make this Declaration on its behalf.

We, the said Raymond P. Ohlmuller

and Jussi J. Saukkonen, M.D.

do solemnly and sincerely declare as follows:

1. The said Becton Dickinson & Company and Thomas Jefferson University are the applicants for the patent.

2. The basic application as defined by Section 141 of the Act was made in United States of America on 24 May 1989 by Stuart K Williams, Bruce E Jarrell, Deborah G Rose, Paul G Alchas, Frank A Augello, Christopher J Brooks, Tony A Cutshall, Joseph A DiPisa Jr., Jonathan B Gabel, Paul J Mulhauser and Wes Prais

3. Stuart K Williams of 103 Cambridge Drive, Wilmington, Delaware; Bruce E Jarrell of 8101 St. Martins Lane, Philadelphia, Pennsylvania; Deborah G Rose of 1099 Lincoln Court, Warrington, Pennsylvania; Paul G Alchas of 29 Ponds Circle Road, Wayne, New Jersey 07470; Frank A Augello of 5 Old Farm Road, Cedar Knolls, New Jersey 07927; Christopher J Brooks of 10 Gervais Street, Glen Cove, New York 11542; Tony A Cutshall of 60 Harrison Street, Boonton, New Jersey 07005; Joseph A DiPisa Jr. of 84 Maryann Lane, Wyckoff, New Jersey 07481;

Jonathan B Gabel of 17-B Addison Place, Clifton, New Jersey 07012;
Paul J Mulhauser of 67 East 11th Street, New York, New York 10003, and
Wes Prais of 245 Awasting Road, Hewitt, New Jersey 07421,
all in United States of America
are the actual inventors of the invention and the facts upon which the said applicants are entitled to make the application are as follows:-

The said applicants are the assignees of the actual inventors.

4. The basic application referred to in paragraph 2 of this Declaration was the first application made in a Convention country in respect of the invention the subject of the application.

DECLARED at Franklin Lakes this 25th day of July 1990

Becton Dickinson & Company

By: .....

R. P. Ohlmuller
Vice President and Secretary

DECLARED at Philadelphia, PA this 31st day of July 1990

Thomas Jefferson University

.....

Jussi J. Saukkonen, M.D.
Dean, College of Graduate Studies

TO: THE COMMISSIONER OF PATENTS
AUSTRALIA
S&F REF: 130678

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(54) Title
ENDOTHELIAL CELL PROCUREMENT AND DEPOSITION KIT

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356431	**24.05.89**	**US UNITED STATES OF AMERICA**

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(56) Prior Art Documents
US 4908013
US 4820626
US 4736850

(57) Claim

1. An endothelial cell procurement and deposition apparatus for collecting fat from a patient, processing said fat to produce an endothelial cell deposition product, and depositing said product on the surface of a graft, all under sterile conditions established and maintained within the apparatus, characterized by a fat collection means for collecting fat from a patient, sealed digestion means connected to said fat collection means through a closed line to maintain sterility during reception of said fat within said digestion means and for retaining said fat under sterile conditions during rinsing and digestion to produce a digested product, sealed endothelial cell isolation means connected to said digestion means through a closed line for maintaining sterile conditions during reception of said digested product within said cell isolation means and for separating and isolating microvessel endothelial cells from said digested product to produce an endothelial cell product, sealed cell deposition means connected to said isolation means through a closed line for maintaining sterile conditions during reception of said endothelial cell product within said deposition means and for depositing said cells on the surface of a graft to be implanted in a patient and facilitating implantation of said endothelialized graft into a patient, and means for creating pressure differentials through said lines for conveying product between said collection and processing means.

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9. A method for sterilely procuring and depositing endothelial cells onto a graft to be implanted in a patient characterized by the steps of collecting fat from a patient into a sterile fat collection means, sterilely transferring said collected fat through a closed line into a sterile digestion means, rinsing and digesting said fat within said digestion means to produce a digested product, sterilely transferring said digested product through a closed line into a sterile isolation means, isolating endothelial cells from said digested product within said isolation means to produce an endothelial cell product, sterilely transferring said endothelial cell product through a closed line into a sterile cell deposition means, sterilely depositing said cell product onto a surface of a graft to be implanted into a patient, and performing said various transferring steps by establishing pressure differentials through said lines.

637100

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FORM 10

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

COMPLETE SPECIFICATION

(ORIGINAL)

FOR OFFICE USE:

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Complete Specification Lodged:
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Published:

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Related Art:

Name and Address of Applicant:

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Complete Specification for the invention entitled:

Endothelial Cell Procurement and Deposition Kit

The following statement is a full description of this invention, including the best method of performing it known to me/us

5845/3

ENDOTHELIAL CELL PROCUREMENT AND DEPOSITION KIT

A B S T R A C T

The invention is an endothelial cell procurement and deposition kit for collecting fat from a patient, processing said fat to produce an endothelial cell deposition product, and depositing said product on the surface of a graft, all under sterile conditions established and maintained within the components of said kit comprised of: fat collection means for collecting subcutaneous fat from a patient; digestion means connectable to said fat collection means to maintain sterility during reception of said fat and for retaining said fat under sterile conditions during rinsing and digestion to produce a digested product; endothelial cell isolation means connectable to said digestion means for maintaining sterile conditions during reception of said digested product and for separating and isolating microvessel endothelial cells from said digested product to produce an endothelial cell product; cell deposition means connectable to said isolation means for maintaining sterile conditions during reception of said endothelial cell product and for depositing said cells on the surface of a graft to be implanted in a patient and facilitating implantation of said endothelial graft into a patient.

ENDOTHELIAL CELL PROCUREMENT AND DEPOSITION KIT

While autologous vein remains the graft of choice, advanced vascular disease and prior surgical intervention limit the availability of autologous grafts. The use of synthetic grafts provides a means for restoring blood flow to ischemic areas when no alternative is available. Commercially available grafts are far from ideal due to their inherent thrombogenicity. 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 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 lumenal surface. Tissue culture advances have also made the generation of large 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 cells requires the use of undefined media, not easily applicable to the clinical setting.

To overcome the problems associated with seeding large vessel endothelial cells on prosthetic grafts, methods for the isolation of microvessel endothelial cells from autologous adipose tissue followed by high density seeding of a vascular prosthesis were developed.

Although microvessel endothelial cells have been shown to be capable of endothelializing a blood-contacting surface, methods of procuring and depositing these cells in an operating room setting present special considerations. Methods currently used employ standard laboratory equipment such as beakers, flasks, centrifuge tubes, shaker baths, pipettes, syringes, sterile hoods. For example, in Williams' and Jarrell's Patent No. 4,820,626 and related applications, methods of treating a graft surface with endothelial cells are disclosed. According to those methods, subcutaneous adipose tissue is aspirated via a cannula and transferred by vacuum into a mucous trap. The trap is then transferred to a sterile hood for further processing. Adipose tissue is 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

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 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 a syringe, the cells are forced into the lumen of the graft. The graft is manually rotated for 2 hours.

In spite of these advances, a need still exists for a simple, reliable method of producing endothelial cell coatings on a graft in an operating room setting.

The present invention provides a simple, reliable kit for producing an endothelialized graft using microvascular endothelial cells harvested from the patient who is to receive that graft. The subject kit is designed to isolate endothelial cells from human fat, to process that fat to produce a cell deposition product, and to deposit that product on the surface of a graft, all under sterile conditions established and maintained within the components of the kit. The kit is a closed system which lessens the

likelihood of contamination and reduces the amount of labor required and user error.

Accordingly, a primary object of the present invention is the provision of a kit for producing endothelialized grafts for implantation in humans.

Another object of the present invention is the provision of a system which establishes and maintains sterility of harvested autologous endothelial cells during processing procedures required to produce the implantable endothelialized vascular graft.

These and other objects of the present invention will become apparent from the following, more detailed description and is illustrated in its specific embodiment in the accompanying drawings.

According to a first embodiment of this invention, there is provided an endothelial cell procurement and deposition apparatus for collecting fat from a patient, processing said fat to produce an endothelial cell deposition product, and depositing said product on the surface of a graft, all under sterile conditions established and maintained within the apparatus, characterized by a fat collection means for collecting fat from a patient, sealed digestion means connected to said fat collection means through a closed line to maintain sterility during reception of said fat within said digestion means and for retaining said fat under sterile conditions during rinsing and digestion to produce a digested product, sealed endothelial cell isolation means connected to said digestion means through a closed line for maintaining sterile conditions during reception of said digested product within said cell isolation means and for separating and isolating microvessel endothelial cells from said digested product to produce an endothelial cell product, sealed cell deposition means connected to said isolation means through a closed line for maintaining sterile conditions during reception of said endothelial cell product within said deposition means for depositing said cells on the surface of a graft to be implanted in a patient and facilitating implantation of said endothelialized graft into a patient, and means for creating pressure differentials through said lines for conveying product between said collection and processing means.

According to a second embodiment of this invention, there is provided a method for sterilely procuring and depositing endothelial cells onto a graft to be implanted in a patient characterized by the steps

of collecting fat from a patient into a sterile fat collection means, sterilely transferring said collected fat through a closed line into a sterile digestion means, rinsing and digesting said fat within said digestion means to produce a digested product, sterilely transferring said digested product through a closed line into a sterile isolation means, isolating endothelial cells from said digested product within said isolation means to produce an endothelial cell product, sterilely transferring said endothelial cell product through a closed line into a sterile cell deposition means, sterilely depositing said cell product onto a surface of a graft to be implanted into a patient, and performing said various transferring steps by establishing pressure differentials through said lines.

Figure 1 is a schematic of the fat collection unit which is used to collect fat containing microvascular endothelial cells from the patient to receive the graft, which fat is ultimately collected into a fat collection device;

Figure 2 is a schematic of the digestion unit, wherein the digestion device is shown in association with the fat collection device of fat collection unit of Figure 1, which unit is used

to produce a digestion product which is transferred to the endothelial cell isolation device, also shown in Figure 2;

Figure 3 is a diagram of the endothelial cell isolation unit;

Figure 4 is a diagram of the vascular graft processing unit and the endothelial cell deposition unit illustrating the components which produce the endothelial cell product and which transfer that product for deposition on a vascular graft;

Figure 5 is a cross-section, on a greatly enlarged scale, of the fat collection device of Figure 1;

Figure 6(a) is a longitudinal cross-section, in a greatly enlarged scale, of the digestion device of Figure 2;

Figure 6(b) is a bottom view, in a greatly enlarged scale, of the digestion device of Figure 2;

Figure 6(c) is a top end view, in a greatly enlarged scale of the digestion device of Figure 2;

Figure 7(a) is an enlarged front view of the endothelial cell isolation device of Figure 2;

Figure 7(b) is an enlarged side view of the endothelial cell isolation device of Figure 2;

Figure 8 is a diagrammatic cross section of the process tube assembly, shown in Figure 4 within the endothelial cell deposition unit, which process tube assembly is used to introduce the

endothelial cell product onto the interior surface of the graft lumen;

Figure 9 is an enlarged diagrammatic cross-section of the inner and outer process tubes of the vascular graft processing unit illustrated in Figure 8;

Figure 10 is a greatly enlarged side view of the components of the inner process tube of Figure 9;

Figure 11 is a greatly enlarged side view of the components of the outer process tube of Figure 9;

Figure 12 is a bar graph showing the average endothelial cell density achieved per section of processed graft for the grafts processed using the preferred kit of the present invention and those using prior art methods;

Figure 13 is a scanning electron micrograph of a graft processed with the preferred kit of the present invention.

In accordance with the preferred methods of the present invention, subcutaneous fat is removed from the 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 automatically washed initially to

remove red blood cells and other debris, followed by a controlled collagenase digestion for 20 minutes at 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 kit described herein. Following an incubation period, the graft is ready for implantation into the patient. In paired comparisons between the kit 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 acceptable for subsequent high density seeding (range 5.14×10^6 to 4.24×10^7 cells from 50 ccs of fat) and adherence to the graft surface. The kit 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 kit include 1) closed, sterile fluid path; 2) minimal user input; 3) compatibility with an operating room environment; 4) optimization

of the conditions to a highly reproducible process from patient to patient.

The system consists of five primary subsystems: 1) fat collection unit (see Figure 1); 2) digestion unit (see Figure 2); 3) endothelial cell isolation unit (see Figure 3); 4) vascular graft processing unit (see Figure 4); and 5) endothelial cell deposition unit (see Figure 4).

The fat collection unit (Figure 1) collects subcutaneous fat tissue sample from a patient. The components include: in-flow tubing (12), fat collection device (14), vacuum tubing (15), aspiration cannula (10) and an aspiration pump (18). The aspiration pump (18) is used to suction subcutaneous fat tissue from the patient through the cannula (10) and in-flow tubing (12) and into the fat collection device (14).

The fat collection device is shown in Figure 5. It consists of a cylindrical chamber (54) with two vacuum line ports at the top (59 and 61) and an outlet port (60) at the bottom connected to a two-way stopcock (62). A plunger rod (57) passes through the top of the chamber and is connected to a syringe-like stopper (56). The stopper has two holes through which vacuum line ports (59 and 61) pass. When the plunger is in the "down" position, a flexible rubber diaphragm (58) covers the bottom of the stopper and the holes. When the plunger is in the "up" position, the rubber

diaphragm (58) is pushed away from the bottom of the stopper by the vacuum line ports (59 and 61), thus opening communication between the inside of the chamber and the vacuum lines (12 and 15). In order to use the device, it must be placed in line with the vacuum line of a liposuction system by using the elbow connectors (63 and 65). In addition, the plunger rod must be in the "up" position. During liposuction, the device acts as a catch trap for the fat tissue. After the appropriate amount of fat is collected, the vacuum line elbow connectors (63 and 65) are disconnected and the plunger rod (57) is pushed down. The rubber diaphragm (58) assumes its original position covering and sealing the bottom of the stopper as it forces the fat tissue out of the outlet port. The subject device serves two functions: to collect fat and facilitate transfer to the digestion unit in a sterile manner.

The digestion unit (Figure 2) rinses the fat tissue sample with rinse solution and digests it with the enzyme collagenase. The components include: digestion device (16), waste vessel (32) endothelial cell isolation device (30), digestion stand (17), collagenase solution IV bags/sets (20 and 22), rinse solution IV bags/sets (21 and 24), control box (27) for temperature and fluid transfer controls and system vacuum source, assorted tubing connectors, air filters, valves. The fat tissue is manually transferred from the fat collection device (14) through a closed

line into the digestion device (16). The fat tissue is rinsed therein with rinse solution introduced into the chamber from the rinse solution IV bags/sets (21 and 24). The rinse solution is drained from the chamber into the waste vessel (32) after rinsing is completed. The collagenase solution is then transferred from the collagenase solution IV bags/sets (20 and 22) into the digestion device (16). Digestion of the fat tissue by the collagenase solution occurs while the mixture is agitated with filtered air and heated to 37°C. The digested fat tissue and collagenase solution mixture is then vacuum transferred into the endothelial cell isolation device (30) for further processing.

The digestion device is shown in Figure 6. It consists of a chamber (64) with several inlet ports at the top (66, 67, 68, 69 and 70), one of which contains a filter and is connected to a tube (72) which terminates near the bottom of the chamber. A series of "fingers" (74) is bonded to the end of the tube in a radial fashion. At the bottom of the chamber is a conical mesh filter (76) below which are two outlet ports (80 and 82) and a temperature probe sheath (78). During use, the collected fat tissue is introduced into the chamber (64) through one of the top inlet ports (66) followed by rinse solution (Media 199E, Hanks, saline, PBS or other physiological buffered solution) through another of the inlet ports (67). A vacuum line, connected to another inlet port (68)

causes filtered air to enter through the center port (69) and tube (72) which air bubbles up through the fat mixture creating agitation. The "fingers" (74) serve to distribute the bubbling air to ensure uniform agitation and provide a frictional surface to facilitate break-up of the fat. The rinse solution is then drawn out through the bottom of the mesh and expelled through one of the outlet ports (80) leaving behind fat tissue relatively free of blood. Digestive enzyme solution (collagenase, dispase, trypsin, or other tissue dissociation enzyme) is introduced through another of the top inlet ports (70) followed by agitation by bubbling. Throughout this process, a temperature probe (79) inside the probe sheath (78) monitors the process temperature and sends feedback to an external heat controller within the control box (27). When digestion is complete, the digested fat solution, rich in microvessel endothelial cells, is drawn out through the bottom mesh and expelled through an outlet port (82) for subsequent processing. The mesh (76) retains undigested tissue and large fibrous matter which is discarded with the device. The subject device is a closed system which lessens the likelihood of contamination and reduces the amount of labor and user error.

The endothelial cell isolation unit (shown in Figure 3) separates and isolates the endothelial cells from within the digested fat tissue sample. The components include: centrifuge

(33), centrifuge shields (31), endothelial cell isolation device (30). The endothelial cell isolation device (30) is placed into a centrifuge shield and the assembly is placed into the centrifuge (33). Centrifugation isolates the endothelial cells. The endothelial cell isolation device (30) is then placed in line with the vascular graft processing unit and mounted on the endothelial cell deposition unit.

The endothelial cell isolation device is shown in Figure 7. It consists of a primary chamber (88) tapering to a secondary chamber or ampule (90) having inlet and outlet ports (92 and 94). In line with each port (92 and 94) is a two-position valve (91 and 93). The first position allows communication between the primary and secondary chambers. The second position allows communication between the secondary chamber and the outside port. Each valve (91 and 93) is initially turned to the first position. Digested fat tissue is introduced through the top port (84). The device is then placed into a centrifuge and spun. Centrifugation separates endothelial cells into the ampule (90), the dimensions of which are optimized for isolating a "pellet" of endothelial cells between the two ports. The valves are then turned to the second position isolating the "pellet" from the primary chamber (88) above and packed red blood cells below. The endothelial cell "pellet" may then be flushed out by attaching a pressurized line to the inlet

port (92) or vacuum line to the outlet port (94). The subject device is a closed system which maintains sterility and reduces the amount of labor and user error.

The vascular graft processing unit shown in Figure 4 protects, maintains sterility and facilitates the processing of the graft during handling, pre-wetting and cell deposition. The components include: process tube assembly including an inner and an outer tube (46), graft, vacuum line/trap assembly (44), vortex/mesh assembly (34), autologous serum/media solution IV bags/sets (36 and 38). The graft is mounted within the inner tube of the process tube assembly. The purpose of the outer tube is to maintain sterility of the inner tube. The graft is pre-wetted prior to cell deposition by drawing the autologous serum/media solution from an IV bag, through the vortex/mesh assembly, into the lumen of the graft, and out through the graft wall until all air is purged from the inner tube of the process tube assembly. The graft processing unit is then transferred to the endothelial cell deposition unit.

The fully assembled process tube is shown in Figure 8. It consists of two major assemblies: inner process tube (100) and outer process tube (112) (see Figure 9). As shown in Figure 10, the inner process tube consists of the following sub-assemblies: vent cap (104), handle cap (108), inner process tube body (102), tunneler (110), tunneler tip (106). A graft is threaded through

the lumen of the tunneler (110) and is attached to the handle cap (108) prior to assembly. As shown in Figure 11, the outer process tube consists of the following subassemblies: outer process tube body (113), inflow endcap (116), outflow endcap (114). In its fully assembled form, the process tube assembly serves the following functions: it houses, protects and maintains sterility of the graft during shipment and handling in the operating room; it supports the graft and allows fluid access to the graft lumen during endothelialization; it breaks down into a sub-assembly which facilitates implantation of the graft while protecting the endothelial lining. During endothelialization, the inflow endcap of the device (116) is connected to a container of endothelial cell suspension, and the outflow endcap (114) is connected to a vacuum source in the control box (27). Negative pressure external to the porous graft causes the endothelial cell suspension to flow into the graft lumen and out through the wall thereby filtering endothelial cells onto the inner graft wall. The filtered solution continues to flow out through the holes (111) in the tunneler wall (110) and out of the vent cap (104). During this operation, the device may be rotated about its central axis by the addition of rotary fittings at the outer process tube end caps. After endothelialization is complete, the inner process tube (100) is removed from the outer process tube (112) and the handle cap

(108)/tunneler (110)/tip (106) assembly is removed from the inner process tube body (102). The graft may then be "tunneled" through, for example, the patient's leg tissue for proper graft placement without contacting or disturbing the graft. Once positioned, the handle cap (108) is detached from the tunneler (110) and the tunneler (110) is withdrawn, leaving the graft in place for the distal anastomosis. An IV line containing autologous serum media solution may be connected to the handle cap (108) to maintain wetting of the graft lumen during surgical placement. When the distal anastomosis is completed, the graft is snipped at the proximal end, releasing it from the handle cap (108) and readying it for the proximal anastomosis.

The endothelial cell deposition unit shown in Figure 4 promotes endothelial cell deposition onto the lumen of the graft. The components include: process tube rotation fixture (48), insulated trough (50), heating pad (52), water circulator/heater (53). The process tube assembly (46) is positioned on the rotation fixture within the insulated trough and wrapped in the heating pad which is heated by the water circulator. The cell deposition procedure is initiated by using vacuum to draw autologous serum/media solution and the isolated endothelial cells from endothelial cell isolation device (30). The endothelial cells and autologous serum/media solution pass through the vortex/mesh

assembly (34) which breaks up the endothelial cell pellet and filters out gross particulate. The endothelial cells resuspended in the solution are pressurized into the lumen of the graft. The graft filters the solution leaving endothelial cells on the luminal wall. During pressurization, and subsequent cell-graft association, the graft is rotated about its central axis at a constant rate and maintained at 37°C.

Ancillary items include: blood collection bag and transfer bag without anticoagulant to be used for blood collection and serum separation, the serum to be used for the make-up of autologous serum/media solution and an additional solution IV bag filled with autologous serum/media solution and an administration set to be used to maintain the cells during graft implantation.

EXAMPLE 1

Microvascular endothelial cells were isolated and deposited on 4mm x 80cm expanded polytetrafluoroethylene (ePTFE) grafts using both the kit and patented methods. After a two hour rotation, the grafts were rinsed with media and cut into 8 sections. P1 is where the cells were introduced and P8 is the opposite end. The graft segments were hematoxylin stained and the cells counted using an automated image analysis system. Figure 12 provides the average

cell density achieved per section on such Gore-Tex® tubular grafts.

EXAMPLE 2

Endothelial cell product was prepared and deposited on an ePTFE graft using the kit. A scanning electron micrograph of the microvascular endothelial cells deposited on the graft is shown in Figure 13. The endothelial cell product was consistently deposited along the entire length of the graft with no significant variation in cell concentration.

As seen from the above a simple, reliable kit for producing an endothelialized graft using microvascular endothelial cells is provided. These cells are harvested from a patient who is to receive the graft and processed through the use of kit which isolates those cells to produce cell deposition product, and deposits that product on the surface of a graft, all under sterile conditions established and maintained within the components of the kit.

While the foregoing description has been directed to the preferred embodiment kit of the present invention, those of ordinary skill in the art in this field will appreciate that various modifications can be made in the materials and methods described herein without departing from the scope of the present invention, which is defined more particularly in the claims appended hereto.

The claims defining the invention are as follows:

1. An endothelial cell procurement and deposition apparatus for collecting fat from a patient, processing said fat to produce an endothelial cell deposition product, and depositing said product on the surface of a graft, all under sterile conditions established and maintained within the apparatus, characterized by a fat collection means for collecting fat from a patient, sealed digestion means connected to said fat collection means through a closed line to maintain sterility during reception of said fat within said digestion means and for retaining said fat under sterile conditions during rinsing and digestion to produce a digested product, sealed endothelial cell isolation means connected to said digestion means through a closed line for maintaining sterile conditions during reception of said digested product within said cell isolation means and for separating and isolating microvessel endothelial cells from said digested product to produce an endothelial cell product, sealed cell deposition means connected to said isolation means through a closed line for maintaining sterile conditions during reception of said endothelial cell product within said deposition means and for depositing said cells on the surface of a graft to be implanted in a patient and facilitating implantation of said endothelialized graft into a patient, and means for creating pressure differentials through said lines for conveying product between said collection and processing means.

2. Apparatus according to claim 1, characterized by fat receiving means connected to said fat collection means through a closed line for receiving said collected fat and for storing said fat under sterile conditions until needed.

3. Apparatus according to claim 2, characterized in that said fat receiving means further comprises a chamber having a top and a bottom, stopper means in sealing engagement with the chamber and capable of being slidably raised and lowered within said chamber, inlet tube means connected to said fat collection means extending through said stopper means into said chamber, vacuum inlet tube means extending through said stopper means into said chamber, plunger means connected to said stopper means for lowering and raising said stopper means, diaphragm means affixed to said stopper means for sealing said chamber during fat

transfer, said diaphragm means being deflected by said inlet tube means and said vacuum inlet tube means when said stopper means is in a raised position and closing off both said inlet means when the stopper means is in a lowered position thereby facilitating fat transfer from the chamber, and outlet means affixed to the bottom of the chamber including a valve means for regulating the transfer of fat from the chamber.

4. Apparatus according to any of claims 1 to 3, characterized in that the digestion means comprises a chamber having a top and a bottom, a first inlet port affixed at the top of the chamber connectable to the fat collection means for introducing collected fat into the digestion means chamber, a second inlet port affixed at the top of the chamber for introducing digesting solution into the digestion means chamber, a third inlet port affixed at the top of the chamber for introducing rinsing solution into the digestion means chamber, a fourth inlet port affixed at the top of the chamber connectable to a vacuum source, a fifth inlet port affixed at the top of the chamber for introducing filtered ambient air into the digestion means chamber, a tube centrally disposed within the chamber parallel to the longitudinal axis of the chamber, said tube having a top and a bottom and connected at its top end to said fifth inlet port and connected at its bottom end to an agitating means through which tube filtered ambient air can be introduced into said chamber, filtering means connected under the agitating means for retaining undigested debris, monitoring means connected at the bottom of the chamber for monitoring the reaction within the chamber, a first outlet port affixed at the bottom of the chamber for removing rinse solution and digestion debris, and a second outlet port affixed at the bottom of the chamber connected to the endothelial cell isolation means for transferring said digested product to the endothelial cell isolation means.

5. Apparatus according to any of claims 1 to 4, characterized in that the endothelial cell isolation means comprises a first chamber connected to the digestion means for receiving digested product and having a top and a bottom, a first inlet port at the top of said first chamber connected to the digestion means, a second chamber connected to the bottom of the first chamber to isolate the

endothelial cell product, a second inlet port affixed to said second chamber having a two-position valve allowing communication between said first and second chamber when in a first position and isolation between said chambers when in a second position, an outlet port affixed to said second chamber having a second two-position valve allowing closing and opening said outlet port in first and second positions, respectively, of said second valve, whereby when said isolation means containing said digested product is centrifuged, the endothelial cells are isolated from the digested product within said second chamber when both of said two position valves are in said second position.

6. Apparatus according to any of claims 1 to 5, characterized in that the cell deposition means comprises an inner process tube assembly having an inlet and outlet means for supporting the graft during deposition of endothelial cell product onto the graft under sterile conditions, and an outer process tube assembly for housing the inner process tube assembly and maintaining sterility during the cell deposition process.

7. Apparatus according to claim 6, characterized in that the outer process tube assembly further comprises a tube body, a first endcap connected to the tube body having an inlet means connected to the endothelial cell isolation means and a communicating outlet means connected to the inlet means of the inner process tube assembly, and a second endcap connected to the tube body having communicating inlet and outlet means, said second endcap inlet means being connected to the outlet means of the inner process tube assembly, and rotary fittings on both endcaps whereby the device may be rotated about its central axis.

8. Apparatus according to claim 6 or claim 7, characterized in that the inner process tube assembly comprises a tunneler assembly connected to the tube body at the end thereof which is connected to the cell isolation means, and a vent cap having an outlet means connected to the inlet means of said second endcap, whereby cell product introduced under pressure flows through the graft housed in the tunneler assembly and deposits cell product onto the graft.

9. A method for sterilely procuring and depositing endothelial cells onto a graft to be implanted in a patient characterized by the steps of collecting fat from a patient into a sterile fat collection means, sterilely transferring said collected fat through a closed line into a sterile digestion means, rinsing and digesting said fat within said digestion means to produce a digested product, sterilely transferring said digested product through a closed line into a sterile isolation means, isolating endothelial cells from said digested product within said isolation means to produce an endothelial cell product, sterilely transferring said endothelial cell product through a closed line into a sterile cell deposition means, sterilely depositing said cell product onto a surface of a graft to be implanted into a patient, and performing said various transferring steps by establishing pressure differentials through said lines.

10. An endothelial procurement and deposition apparatus substantially as hereinbefore described with reference to the accompanying drawings.

11. A method for sterilely procuring and depositing endothelial cells according to claim 9 and substantially as hereinbefore described.

DATED this FOURTH day of JANUARY 1993

Becton Dickinson & Company
Thomas Jefferson University
Patent Attorneys for the Applicants/Nominated Persons
SPRUSON & FERGUSON

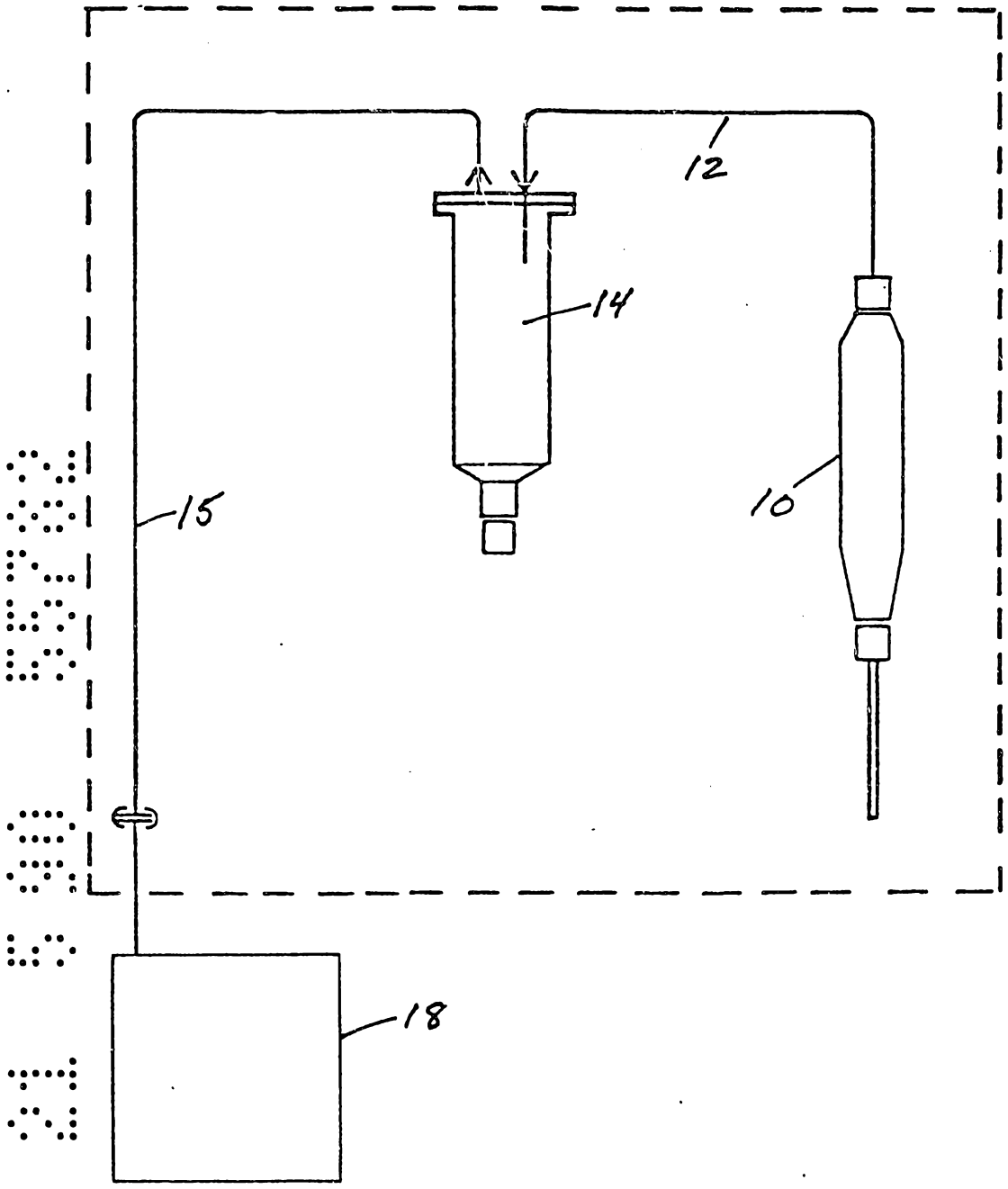


FIGURE 1

FIGURE 2

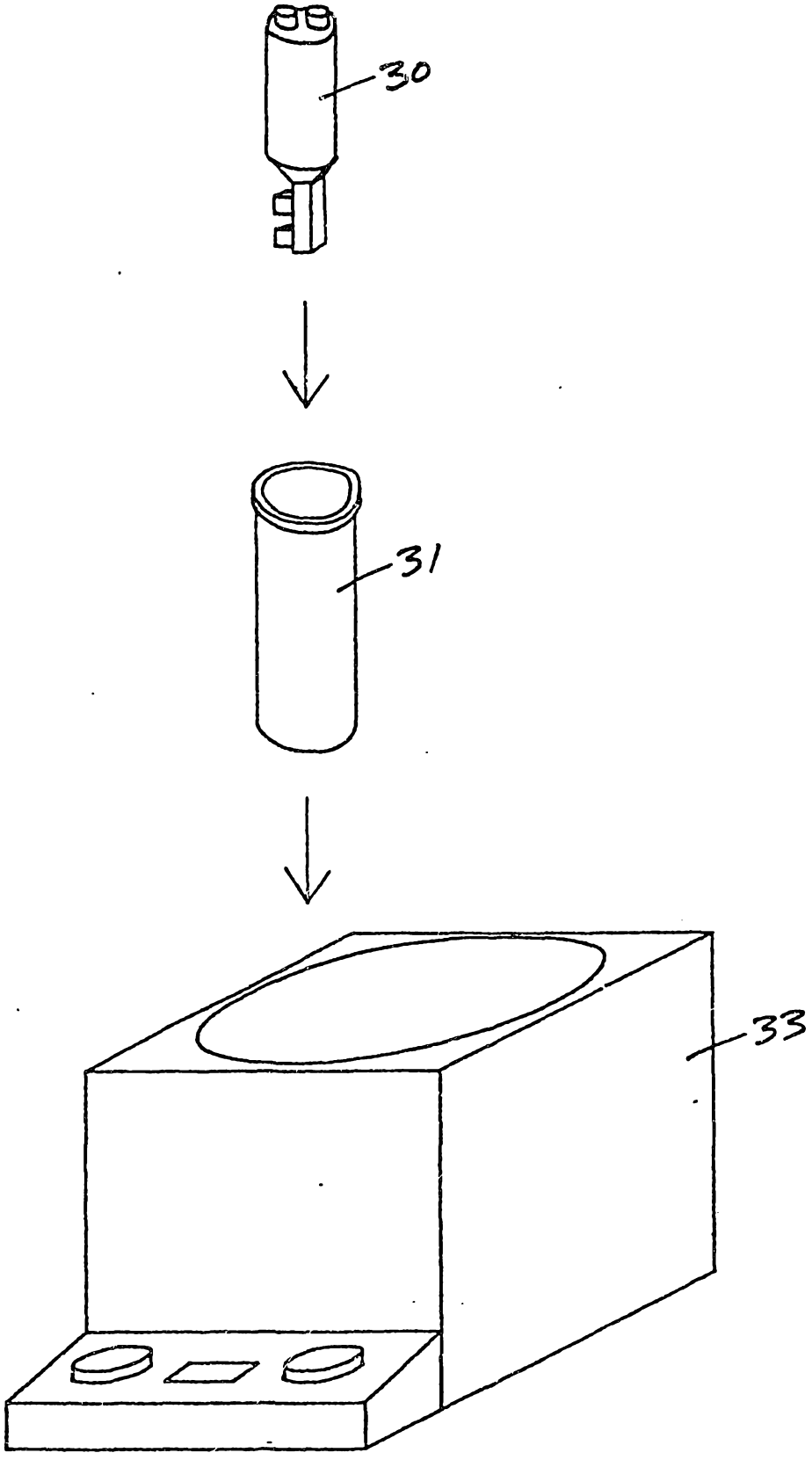


FIGURE 3

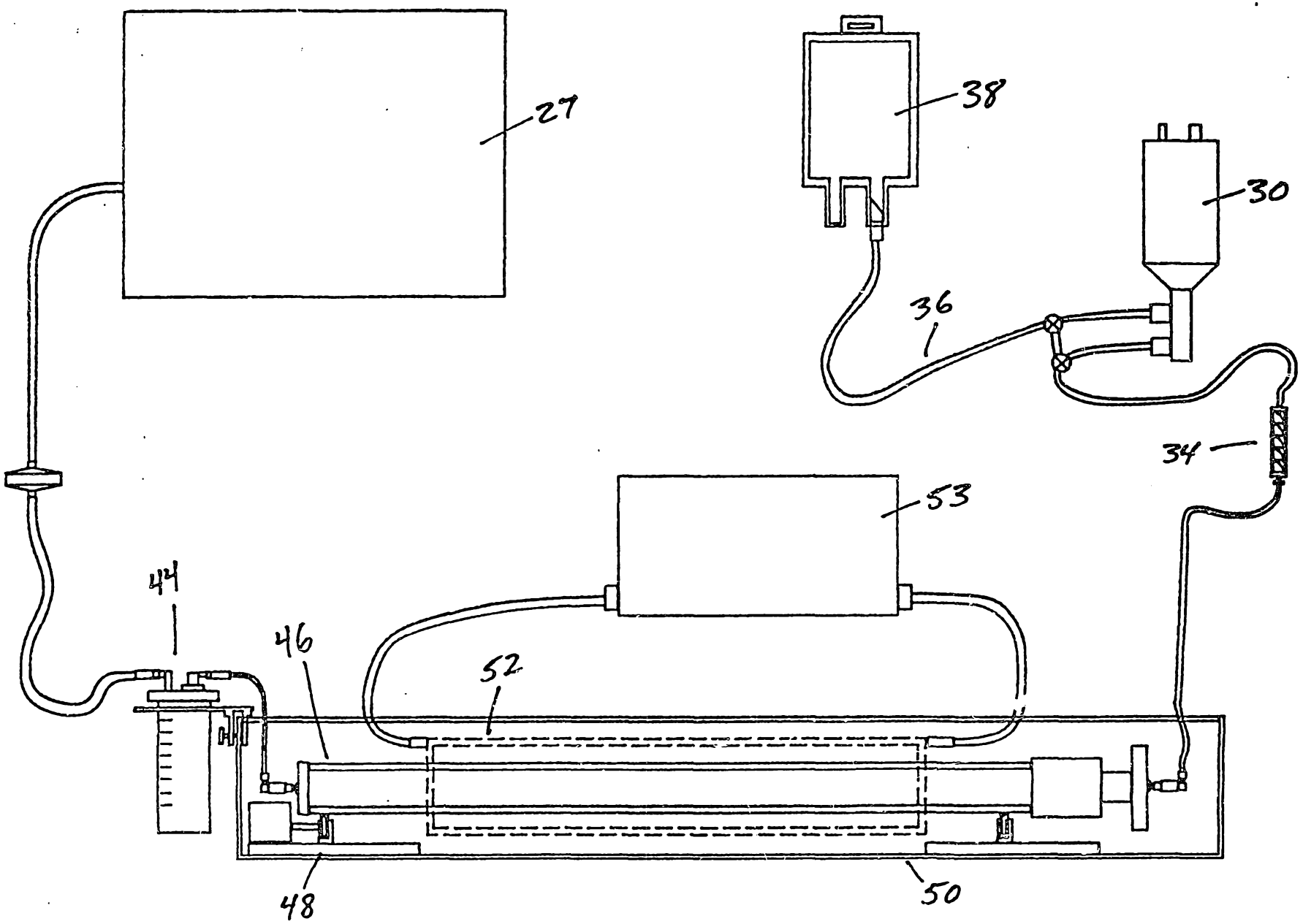


FIGURE 4-

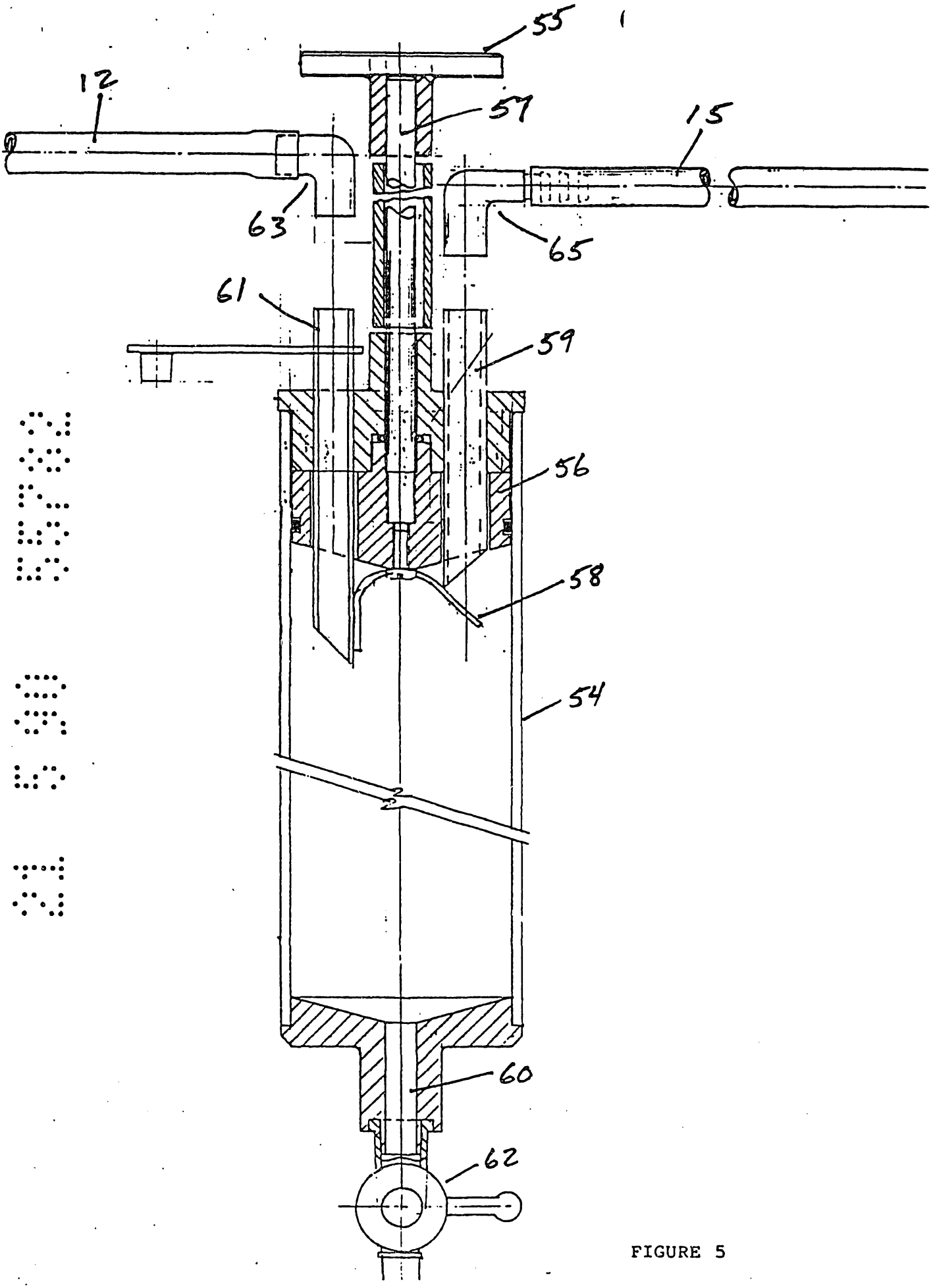


FIGURE 5

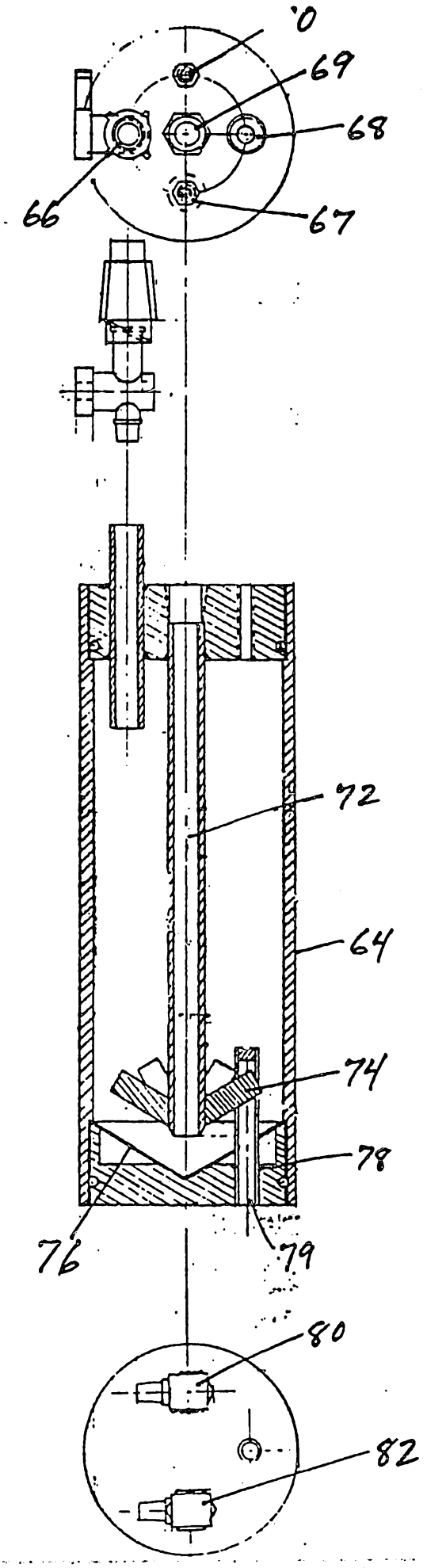


FIGURE 6a, 6b, 6c

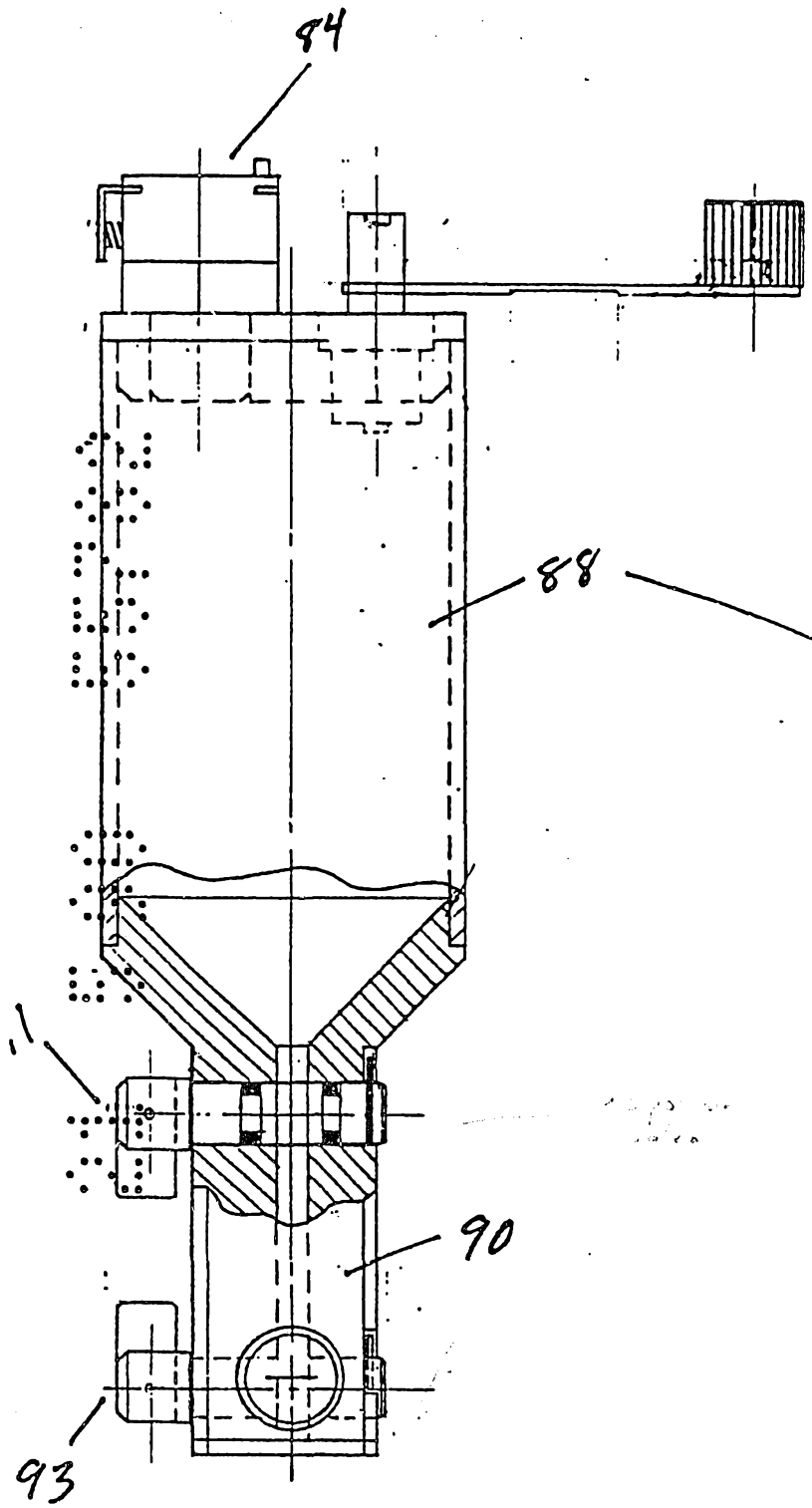


Figure 7 a

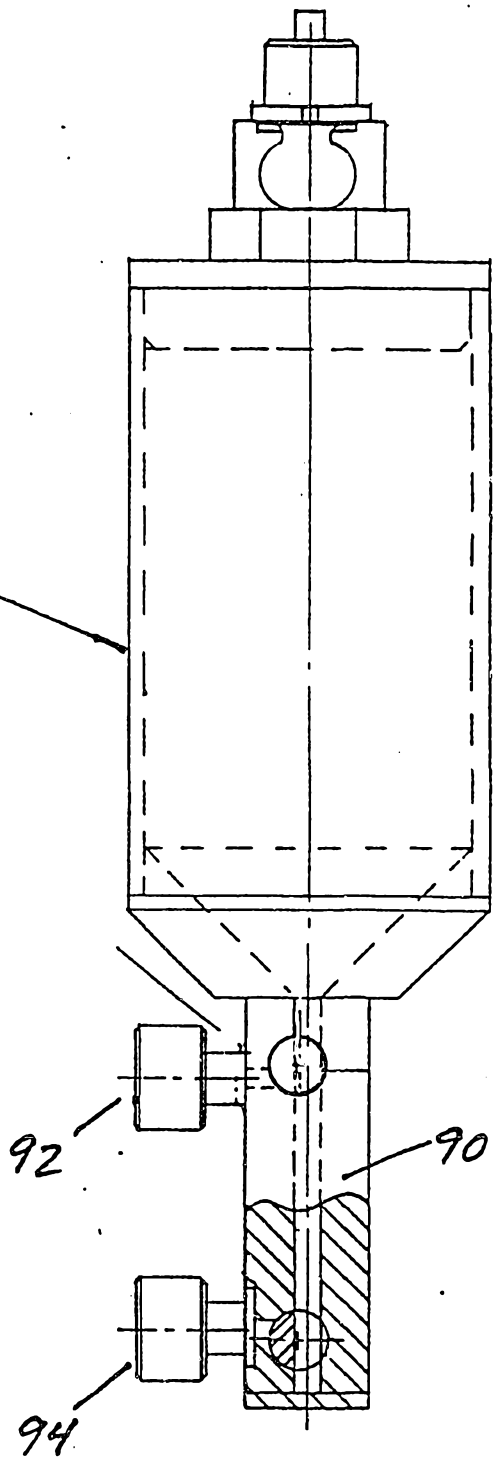


Figure 7 b

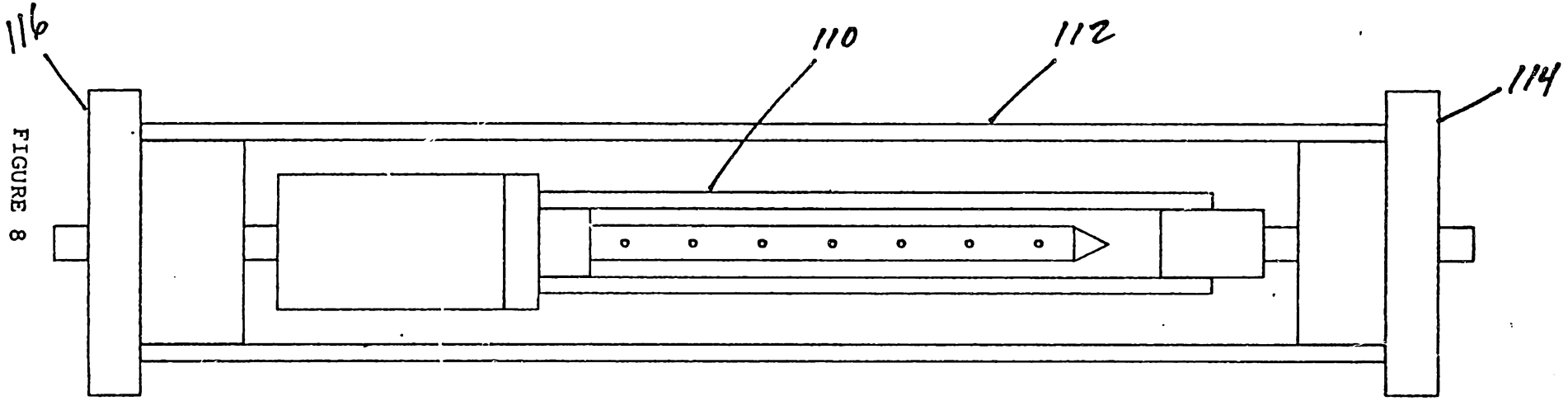
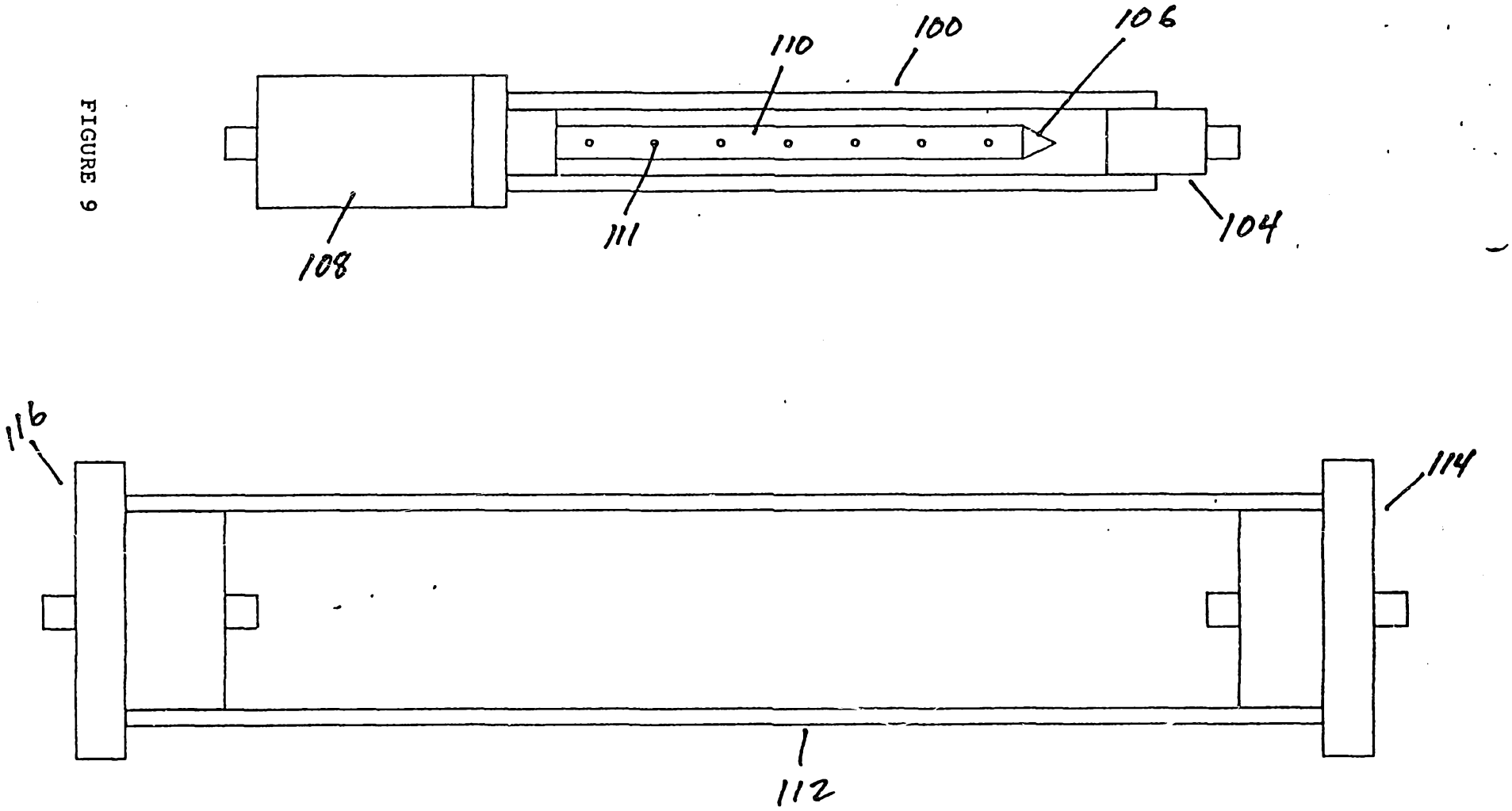


FIGURE 8

FIGURE 9



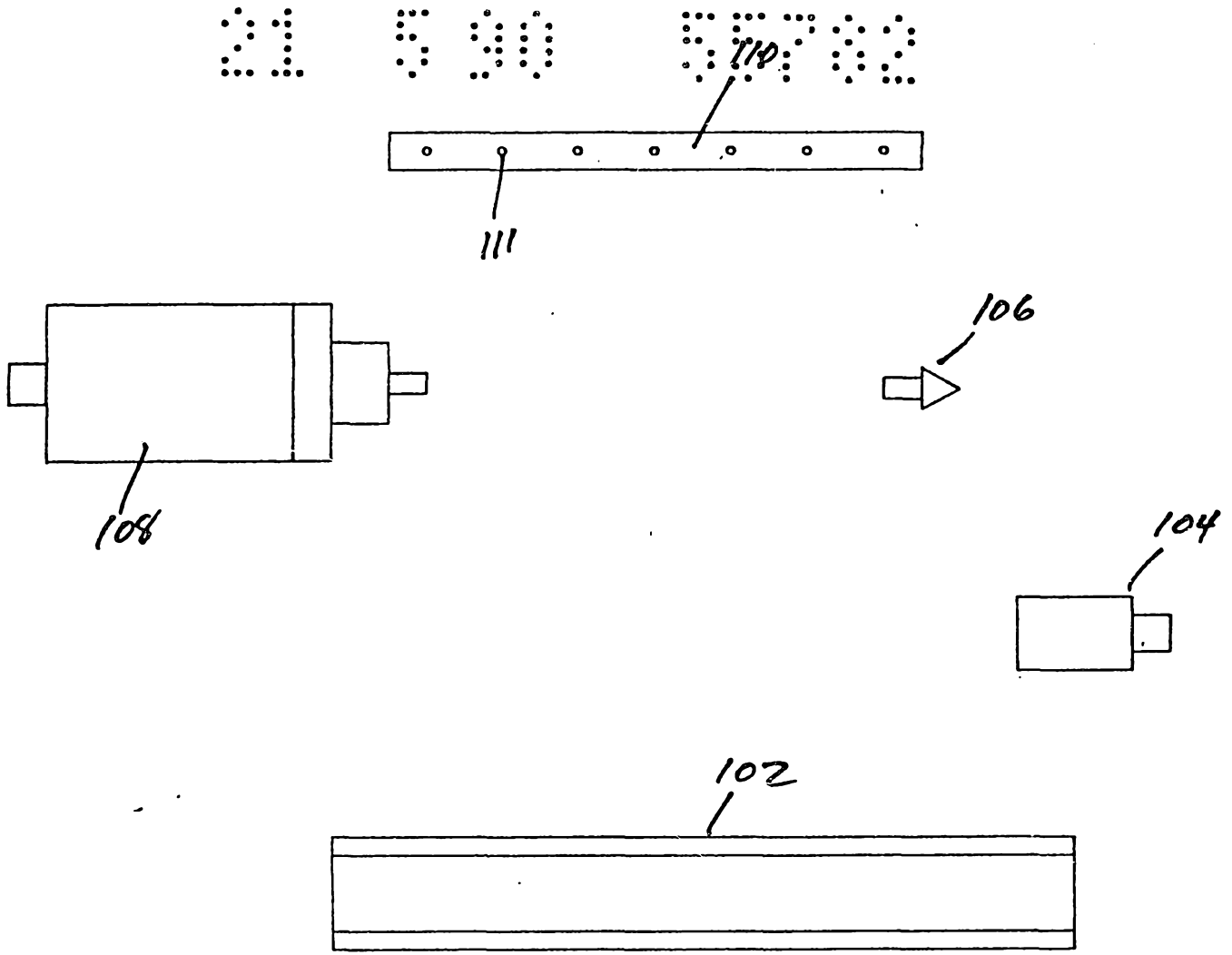


FIGURE 10

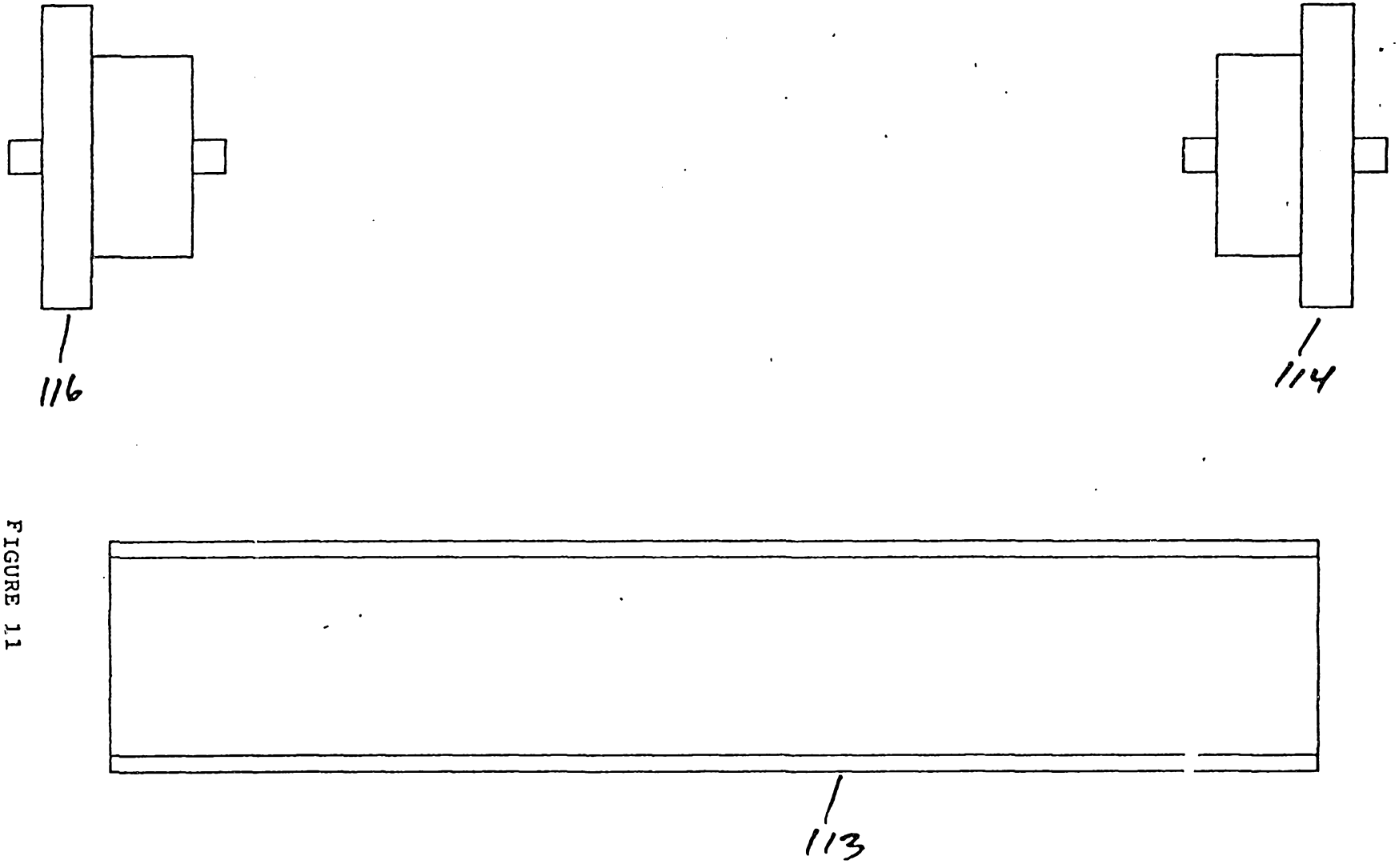
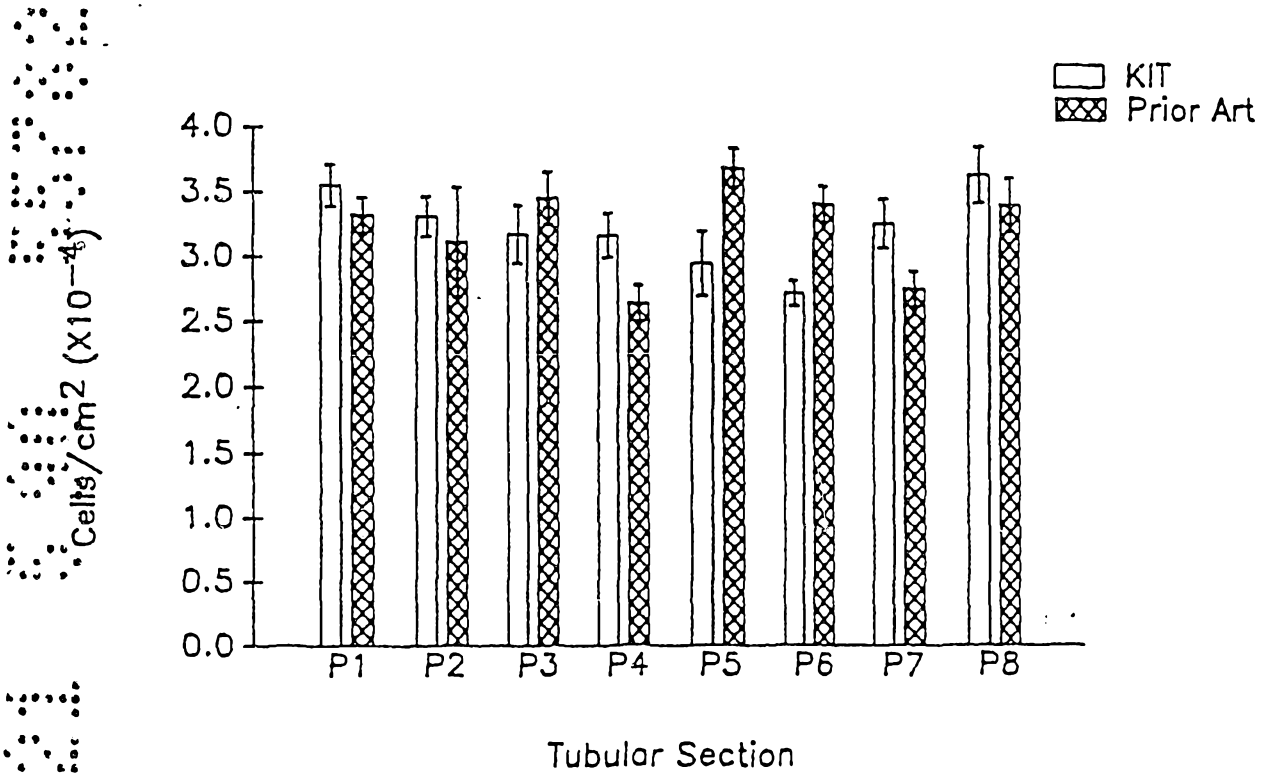


FIGURE 11


KIT
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2.0
1.5
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0.0
P1
P2
P3
P4
P5
P6
P7
P8
Tubular Section

FIGURE 12

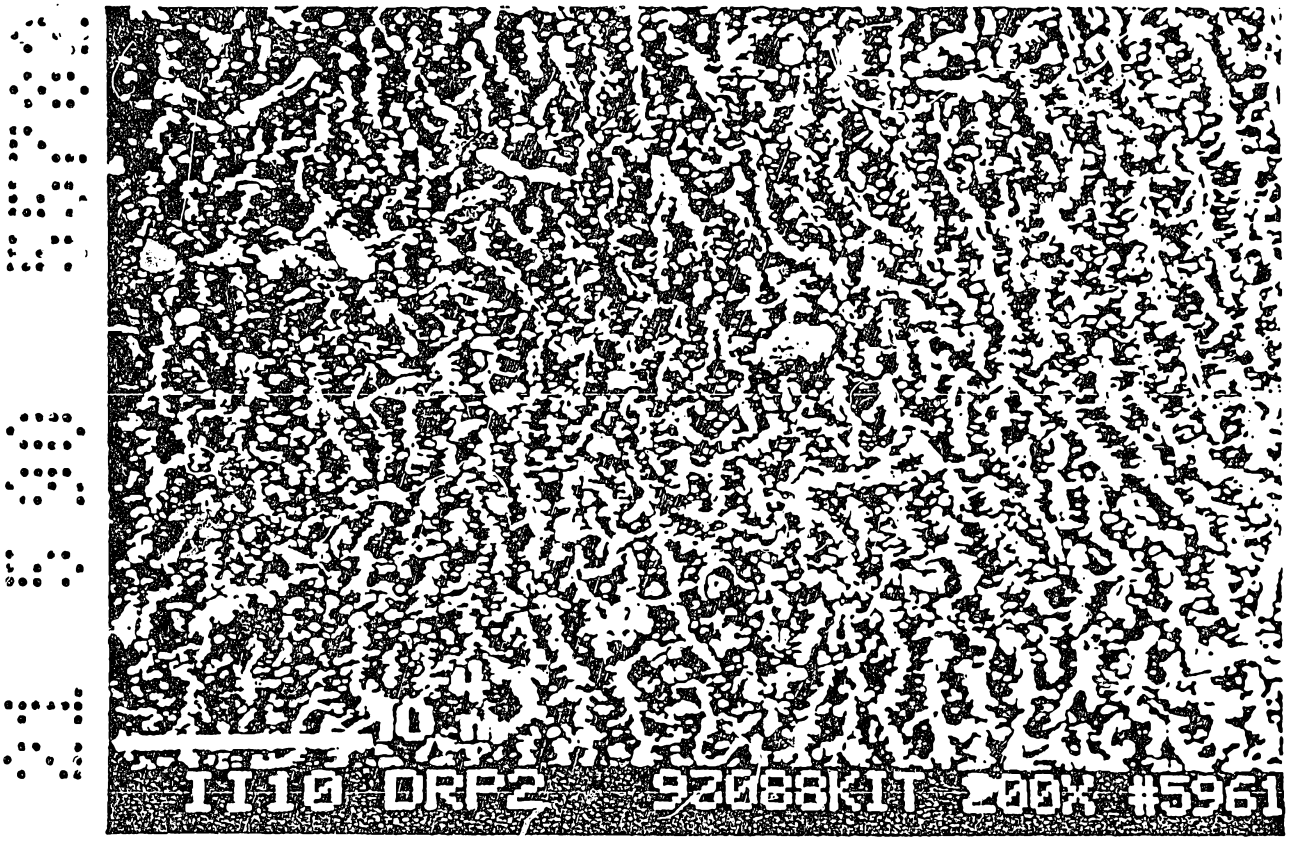

FIGURE 13