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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 September 2005 (22.09.2005)

PCT

(10) International Publication Number
WO 2005/087292 A1

(51) International Patent Classification[7]: A61M 5/00, 5/178, 5/315, B01D 21/26

(21) International Application Number: PCT/US2005/008175

(22) International Filing Date: 11 March 2005 (11.03.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/551,773 11 March 2004 (11.03.2004) US

(71) Applicant *(for all designated States except US)*: **HARVEST TECHNOLOGIES CORPORATION** [US/US]; 40 Grissom Road, Suite 100, Plymouth, MA 02360 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LARIVIERE, Richard** [US/US]; Harvest Technologies Corporation, 40 Grissom Road, Suite 100, Plymouth, MA 02360 (US). **MCGILLICUDDY, Andrew** [US/US]; Harvest Technologies Corporation, 40 Grissom Road, Suite 100, Plymouth, MA 02360 (US). **ELLSWORTH, James, R.** [US/US]; Harvest Technologies Corporation, 40 Grissom Road, Suite 100, Plymouth, MA 02360 (US).

(74) Agent: **CLARK, Conrad, J.**; Clark & Brody, 1090 Vermont Avenue, N.W., Suite 250, Washington, DC 20005 (US).

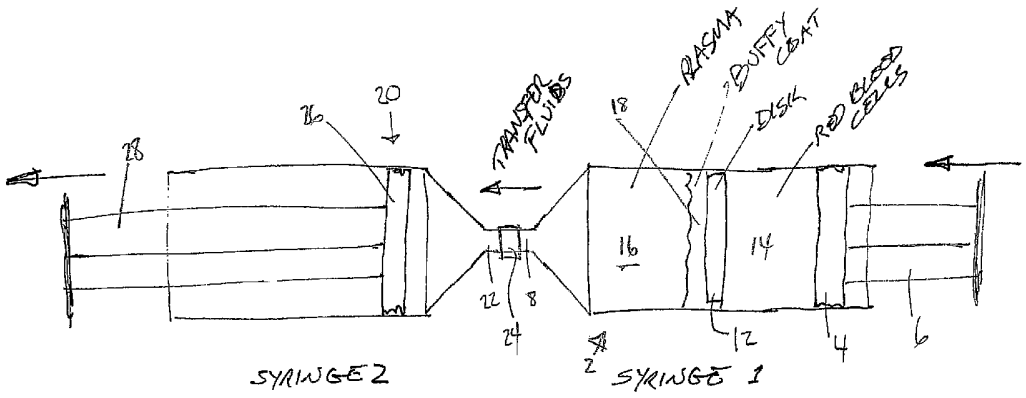
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

[Continued on next page]

(54) Title: METHOD FOR OBTAINING AN INTERMEDIATE FRACTION IN THE CENTRIFUGAL SEPARATION OF BLOOD



(57) Abstract: A method for obtaining an intermediate fraction (18) from a fluid includes the steps of introducing the fluid to a first container (2) and centrifuging the fluid to separate a first fraction (14) from the fluid. The remainder of the fluid is then transferred to a second container (20) and placed in a centrifuge such that the intermediate fraction (18) will form adjacent a discharge port (22) of the second container (20). The intermediate fraction (18) may then be expressed easily. A kit according to the invention includes the two containers.

WO 2005/087292 A1

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

METHOD FOR OBTAINING AN INTERMEDIATE FRACTION IN THE CENTRIFUGAL SEPARATION OF BLOOD

TECHNICAL FIELD

[0001] This invention relates to the art of fluid fractionalization. In particular, this invention relates to a system for obtaining blood fractions of intermediate density.

BACKGROUND ART

[0002] It is known to separate blood centrifugally into several fractions. In the system described in United States Patent 5,707,331, blood is placed in one chamber of a disposable unit that has two adjacent chambers. The disposable unit is then placed in a centrifuge, and the centrifuge operated to separate the blood into fractions. The disposable unit is then tilted to allow lighter fractions to decant into the second chamber. In the preferred embodiment, the lighter fractions comprise plasma and a buffy coat, which includes the platelets and white blood cells. The buffy coat lies at the interface of the lighter plasma and the heavier red blood cells and is, thus, an intermediate fraction. In the Wells system, the plasma and buffy coat fractions that have been decanted into the second chamber are subjected to a second centrifugation whereby the buffy coat is caused to lie at the bottom of the second chamber.

[0003] In the Wells system, the buffy coat can be removed from the second chamber after the second centrifugation by passing a cannula through the upper layer containing only plasma and into the buffy coat and withdrawing the buffy coat into a syringe.

[0004] The above process requires sophisticated hardware capable of performing several complicated steps and also requires significant human interaction in the withdrawing of the buffy coat.

SUMMARY OF THE INVENTION

[0005] In accordance with the invention, fractions of intermediate density are easily separated from a mixture by using two containers, each of which is capable of

expressing one or more fractions in such a manner that the desired fraction of intermediate density can be obtained.

[0006] In the preferred embodiment, whole blood is obtained from a patient, and the desired fraction to be separated from the whole blood is the buffy coat, the fraction of blood that includes platelets, among other components. The buffy coat is obtained from the whole blood by introducing the whole blood into a first container, preferably a first syringe, from which a separated fraction may be expressed into a second container. The first syringe is placed in a centrifuge in an orientation whereby the heavier fraction containing red blood cells is caused to accumulate adjacent the end of the syringe having a plunger. The lighter fractions containing plasma and the buffy coat then accumulate in the region adjacent the input/discharge end of the syringe. It is also preferred that a separator of appropriate density be employed, which separator automatically positions itself at the interface between the buffy coat and the red blood cells and reduces mixing of these layers after centrifugation.

[0007] A syringe is a preferred container because its input/discharge end is conical, which is advantageous during discharge because it provides increased control over discharge of fluids in the region of the interface between plasma and red blood cells. Containers other than a syringe may be employed, such as an evacuated tube that draws fluid into the tube under reduced pressure.

[0008] The first centrifugation of the whole blood is just enough to separate plasma from red blood cells. This first spin provides mainly two separated fractions, platelet rich plasma and red blood cells. As is known, however, intermediate fractions begin to form even during the first spin. Thus, the buffy coat begins to form during this first spin but is not large or well formed. After the first centrifugation of the whole blood, the syringe plunger is actuated to express the platelet rich plasma and the initial layers of the intermediate fractions, including the buffy coat, into a second container, preferably a syringe. This is accomplished

in the preferred embodiment by connecting the input/output ends of the two syringes together with a Luer connector.

[0009] The second syringe is then placed in a centrifuge such that the heavier buffy coat accumulates in the region immediately adjacent the discharge end and the platelet poor plasma accumulates adjacent the plunger. This allows the buffy coat and any desired quantity of plasma to be expressed by actuation of the plunger.

[0010] It will be appreciated that the process is sterile because the syringes are sterile and the fluids are not exposed to the ambient during the process.

[0011] It is therefore an object of the invention to provide a simple, sterile process for the separation of an intermediate fluid fraction by centrifugation.

[0012] It is a further object of the invention to provide a process for obtaining an intermediate fluid fraction by centrifugation, wherein a fluid is placed in a first container, a first fraction containing the intermediate fraction is separated by centrifugation and transferred to a second container, the intermediate fraction is separated from the first fraction by centrifugation such that it is positioned adjacent the outlet of the second container, and the intermediate fraction is then expressed from the second container.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1 is a side view of the first syringe illustrating the first step in a preferred method according to the invention.

[0014] Figure 2A is a side view of two syringes connected to each other at the beginning of a further step in the preferred method according to the invention.

[0015] Figure 2B is a side view of the two syringes shown in figure 2A at the completion of the further step in the preferred method according to the invention.

[0016] Figure 3 is a side view of a second syringe showing a still further step in the preferred method according to the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0017] With reference to the drawings, figure 1 illustrates a syringe 2 into which a quantity of whole blood has been drawn. Syringe 2 may be a known syringe having a plunger 4 and a plunger handle 6 or may be a custom container into which the quantity of blood is placed. In the embodiment shown, the whole blood is drawn into the syringe by moving the plunger away from the tip 8 of the syringe. The tip 8 is preferably attached directly to a needle through which the blood is drawn from the patient. As well, the tip 8 may be inserted into a separate container having blood previously drawn from the patient. It will be appreciated that the syringe 2 includes a conical section 10 adjacent the tip 8, and that the tip 8 is the input/discharge port of the syringe and generally includes a Luer-type connector for detachable connection to another syringe, as will be described below, or to tubes and the like.

[0018] The syringe 2 includes a floating element 12, which is configured and made of material having a density whereby it assumes a position wherein the surface closest the tip lies just below the interface of the platelet rich plasma and the red blood cells after a first centrifugation of the blood. The syringe 2 may be a known syringe modified by addition of the floating element 12.

[0019] In accordance with a first step of a method according to the invention, the syringe 2 having whole blood therein is placed in a centrifuge (not illustrated) such that the forces arising from centrifugation are in the direction illustrated by the "G force" arrow in the figure. The centrifuge is then operated in a "soft spin" to separate the whole blood into a red-blood-cell fraction 14, a platelet-rich-plasma fraction 14, and an initial, thin layer of the buffy coat fraction 18. It will be appreciated that the syringe 2 is placed in the centrifuge in an orientation whereby the plasma layer 16 forms adjacent the input/discharge end of the syringe 2 during centrifugation.

[0020] A known centrifuge may be used for the processes described herein, the only major change being that its diameter be large enough to accommodate the handle 6. Alternatively, the syringe is constructed such that the handle is connected to the plunger by a detachable connector, such as a screw thread, a clip, or the like whereby may be removed or placed in an orientation that does not extend appreciably from the end of the syringe.

[0021] Figures 2A and 2B illustrate a further step of the preferred process according to the invention. As shown in figure 2A, the input/discharge end of first syringe 2 is connected to the input/discharge end of a second syringe 20 by connecting the tip 8 of the first syringe to the tip 22 of the second syringe by a connector 24, e.g., a Luer connector. Then, as illustrated in figure 2B, the plunger 4 of syringe 2 is actuated by pushing on handle 6 to force the plasma 16 and buffy coat 18 into the syringe 20, leaving the red blood cells 14 in syringe 2. It will be appreciated that the initial buffy coat layer may mix with the platelet rich plasma during the transfer illustrated in figures 2A and 2B. The important separation at this stage is between the red blood cells and the remaining fractions, and it may be desirable to intentionally mix the initial buffy coat layer with the plasma, as by shaking the syringe, to ensure that the majority of the cells of the buffy coat are transferred to the second syringe with the plasma.

[0022] The floating disc 12 reduces mixing between the red blood cells and the plasma during handling of the syringe after the first centrifugation. It will be appreciated further that friction with the side of the syringe tends to cause the central portion of the fluid to be expressed first but that the presence of the disc 12 will prevent the red blood cell layer from pushing through the plasma layer during expression of the plasma. Also at the end of the expression of the plasma fraction, the disc 12 will engage the conical end of the syringe 2, and it is preferable that the disc be configured to provide a passage between its periphery and the inside wall of the syringe, e.g., by providing flattened sections on the edge of the disc.

This allows cells to pass in both directions during centrifugation but also allows red blood cells to pass toward the discharge end after engagement between the disc and the conical portion to ensure expression of the entire plasma layer by filling the conical portion with red blood cells. Because the tapered configuration allows control over the expression this is easily accomplished.

[0023] Figure 3 illustrates a further step in the process wherein syringe 20 is placed in a centrifuge in an orientation whereby the buffy coat will accumulate adjacent the input/discharge end 30 of syringe 20. This is accomplished, in essence, by placing syringe 20 in the centrifuge in an orientation that is opposite to the orientation of syringe 2 in the centrifuge. The centrifugal forces on syringe 20 are illustrated in figure 3 by the "G Force" arrow.

[0024] After centrifugation as illustrated in figure 3, a buffy coat layer 18 forms adjacent the discharge tip 22, and this may be expressed along with a desired volume of plasma by actuation of the plunger 26. If the buffy coat is discharged with a desired volume of plasma platelet-rich plasma having a desired increase in concentration over native levels is obtained. .

[0025] Modifications within the scope of the appended claims will be apparent to those of skill in the art.

We claim:

1. A method for separating a blood fraction containing platelets from whole blood comprising the steps of:

introducing said whole blood into a first syringe,

subjecting said first syringe to centrifugation such that plasma accumulates adjacent a discharge end of said first syringe,

transferring said plasma and a buffy coat fraction into a second syringe,

subjecting said second syringe to centrifugation such that said buffy coat accumulates adjacent a discharge end of said second syringe.

2. A method according to claim 1 further comprising the step of expressing said buffy coat.

3. A method according to claim 2 further comprising the step of expressing a predetermined volume of plasma with said buffy coat.

4. A method according to claim 1 wherein said step of transferring comprises connecting a discharge port of said first syringe to an input port of said second syringe.

5. A kit for obtaining an intermediate fraction from a fluid by centrifugation comprising, a first container forming a cavity and having a discharge port for expressing fluids from said cavity, said first container having a floating disc therein for assuming a position during centrifugation near the boundary of first and second fractions of said fluid during centrifugation, a second container having an input port and a discharge port for discharging said intermediate fraction after centrifugation, and a connector for connecting said discharge port of said first container to said input port of said second container.

6. A kit according to claim 5 wherein said first and second containers are syringes.

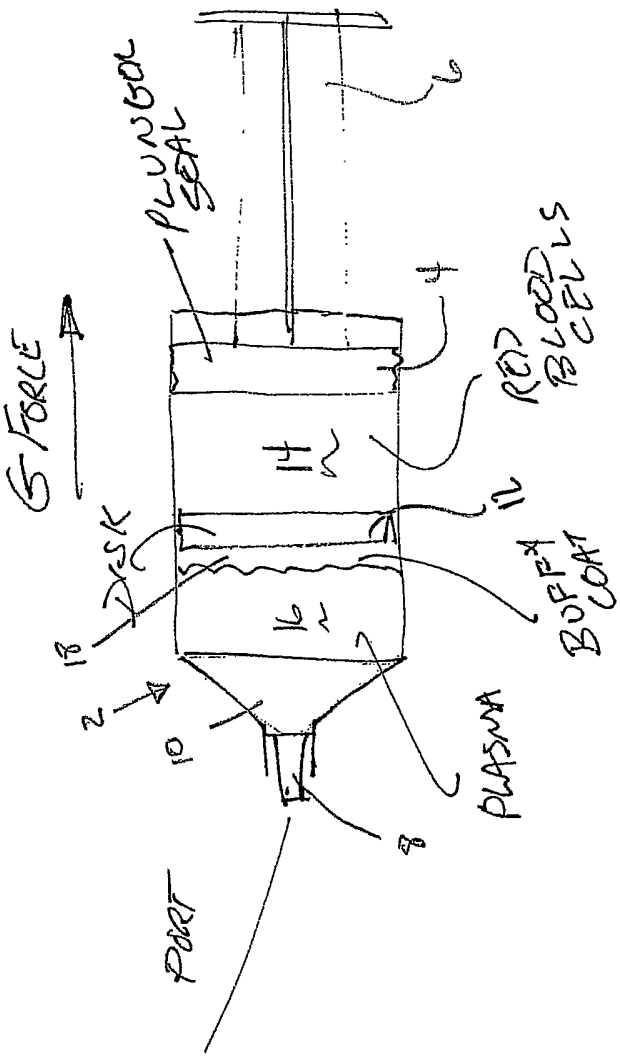


FIGURE 1 SYRINGE 1

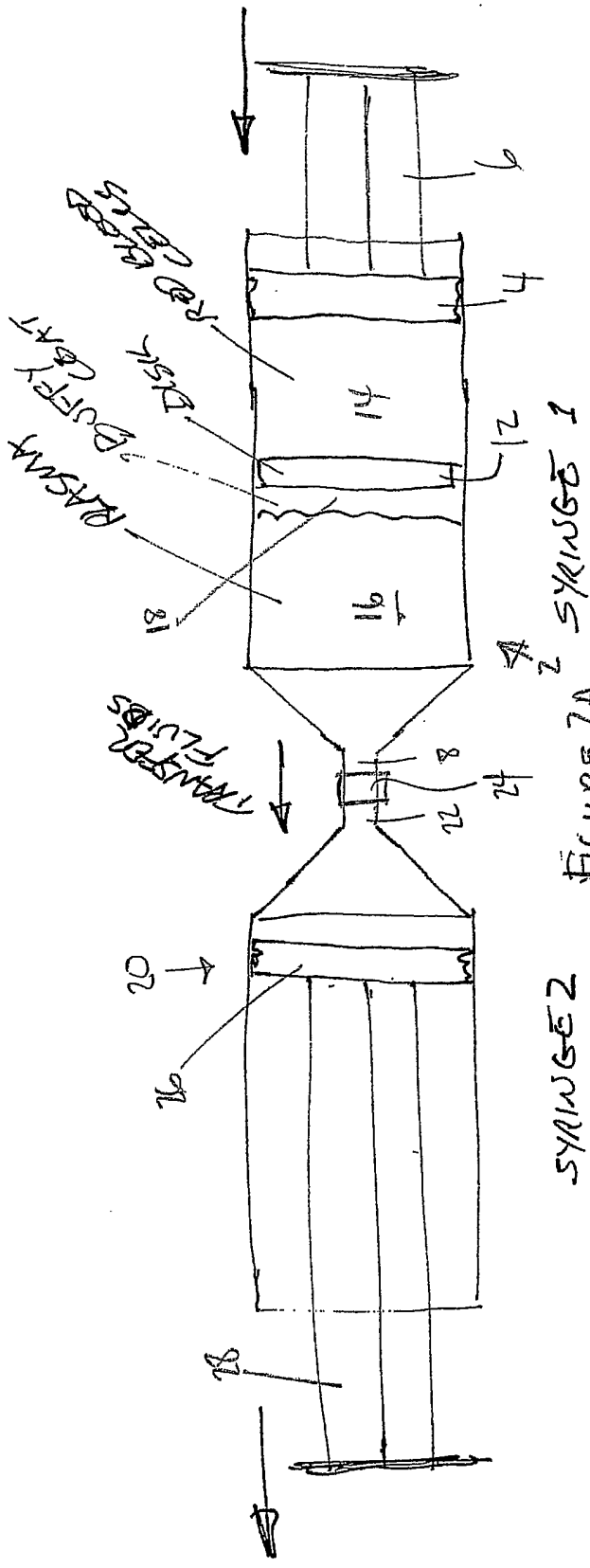


FIGURE 2A

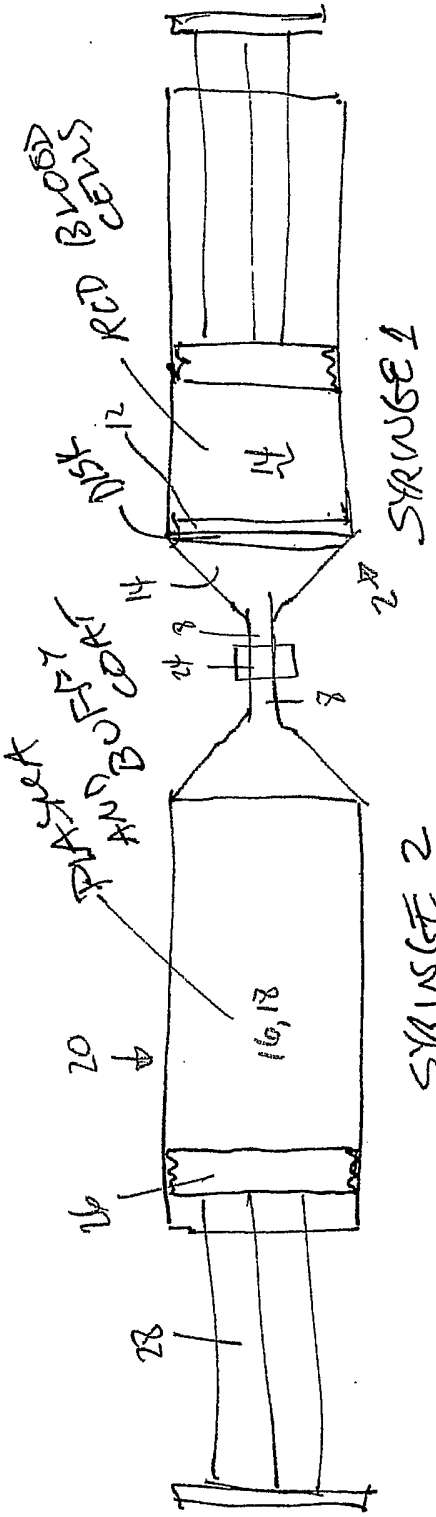


FIGURE 2B

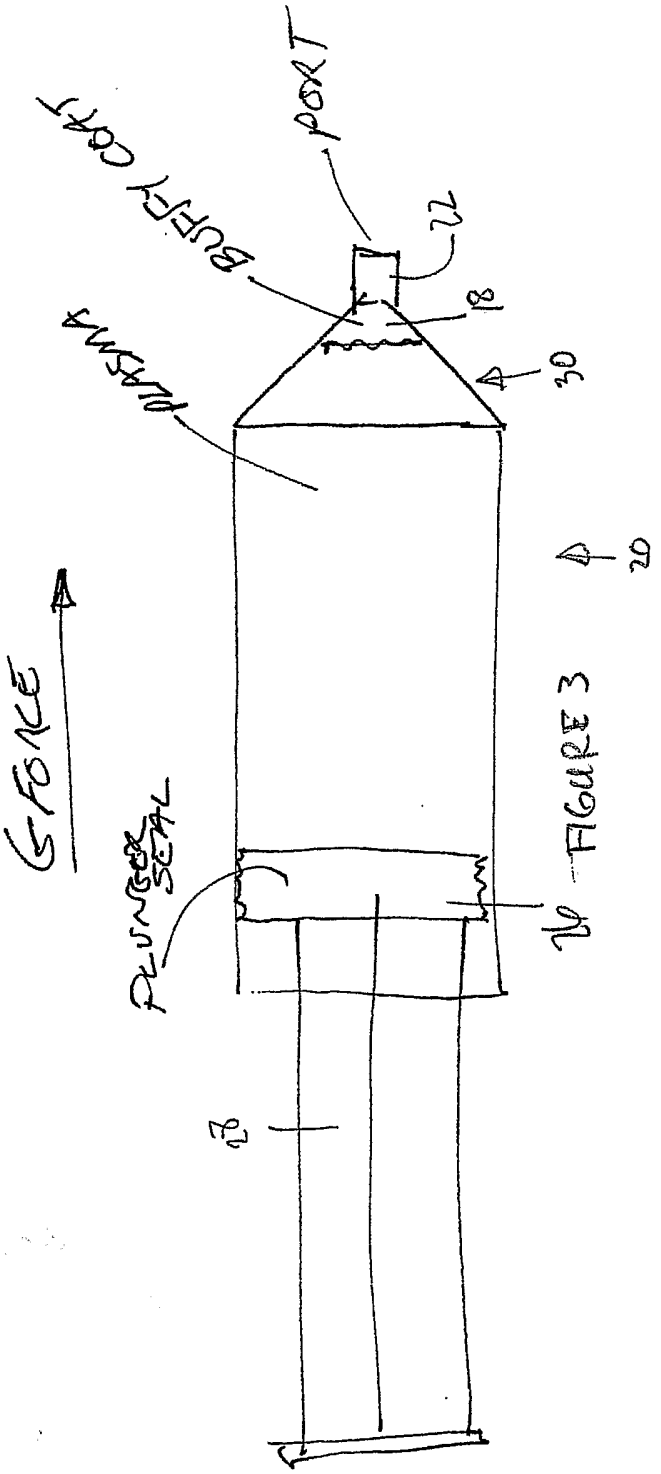


FIGURE 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/08175

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61M 5/00, 5/178, 5/315; B01D 21/26
US CL : 210/782,789,806,252,322,512.1;422/44,72,100,101,102,103,918;436/177,180;604/6.04,6.15,207,211
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 210/782,789,806,252,322,512.1; 422/44,72,100,101,102,103,918;436/177,180;604/6.04,6.15,207,211

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 01/03756 A1 (IMPLNT INNOVATIONS, INC.)18 January 2001 (18.01.2001), page 5, line 12 to page 6, line 20.	1-4 --- 5-6
Y	US 3,972,812 A (GRESL, JR.) 03 August 1976 (03.08.1976), column 2, lines 53-66 and figure 3.	5-6
A	US 6,123,687 A (SIMONYI et al) 26 September 2000 (26.09.2000), entire document.	1-6
A	US 2002/0185457 A1 (SMITH et al) 12 December 2002 (12.12.2002), entire document.	1-6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent 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	Date of mailing of the international search report
23 June 2005 (23.06.2005)	15 JUL 2005
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer David A. Reifsnyder Telephone No. (571) 272-1700

Form PCT/ISA/210 (second sheet) (January 2004)