PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
B01D 39/16, 67/00

A1

(11) International Publication Number: **WO 95/18665**

(43) International Publication Date: 13 July 1995 (13.07.95)

(21) International Application Number: PCT/US95/00355

(22) International Filing Date: 10 January 1995 (10.01.95)

(30) Priority Data:

08/179,567	10 January 1994 (10.01.94)	US
08/204,102	1 March 1994 (01.03.94)	US
08/347,564	30 November 1994 (30.11.94)	US

(71) Applicant: HEMASURE, INC. [US/US]; 33 Locke Drive, Marlborough, MA 01752 (US).

(72) Inventors: CASTINO, Franco; 201 Peakham Road, Sudbury, MA 01766 (US). AZAD, Abdul, R., M.; 10 Potter Circle, Northborough, MA 01532 (US). LEE, Eric, Kin-Lam; 3 Jay Lane, Acton, MA 01720 (US). FOURON, Yves; 44 DeSimone Drive, Marlborough, MA 01752 (US). ZEPP, Charles, M.; 940 North Road, Hardwick, MA 01037 (US).

(74) Agents: HANSEN, Philip, E. et al.; Heslin & Rothenberg, P.C., 5 Columbia Circle, Albany, NY 12203-5160 (US).

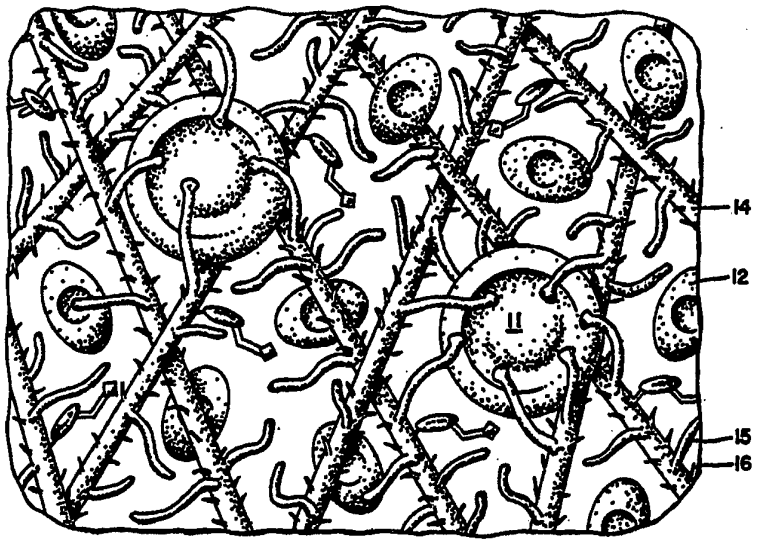
(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: DEVICE AND PROCESS FOR REMOVING LEUKOCYTES AND VIRAL INACTIVATING AGENTS FROM BLOOD



(57) Abstract

Filters (hollow-fibers, flat-sheet membranes or depth filters) for removing leukocytes and viral inactivating agents from whole blood or blood fractions are disclosed. One type of depth filter comprises a laid textile web which has been modified to attach a ligand for viral inactivating agents, a ligand for leukocytes, or ligands for both. An exemplary membrane comprises a polyethersulfone membrane that has been similarly modified. Filter devices for simultaneously removing leukocytes and viral inactivating agents from whole blood or blood fractions are also disclosed. One type of device comprises (1) a housing surrounding (2) activated carbon and (3) a mechanically stable polymeric material which may optionally be modified to attach a ligand for leukocytes. General methods for removing leukocytes and rival inactivating agents from blood are also disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

DEVICE AND PROCESS FOR REMOVING LEUKOCYTES AND VIRAL INACTIVATING AGENTS FROM BLOOD

Field of the Invention

The invention relates to depth filters and membrane filters for removing leukocytes and viral inactivating agents from whole blood or blood fractions, methods for preparing said filters and membranes and methods for using the membranes and filters to remove leukocytes and viral-inactivating agents from whole blood or blood fractions.

Background of the Invention

Blood products, especially those intended for transfusion, are non-homogeneous in that they include several cell types as well as a variety of molecular components having differing biological activities. Often patients into whom the blood is to be transfused are only in need of one component (e.g. red blood cells for gas transport), and the other components present in the blood product are not only unnecessary but may even be disadvantageous or harmful. In this respect, leukocytes have come to be regarded as unwanted passengers in transfusions because "once transfused, they may turn upon their host and unleash endogenous pyrogens, cell-associated viruses, or even lethal graft-versus-host disease." [see Klein "Wolf in Wolf's Clothing: Is It Time to Raise the Bounty on the Passenger Leukocyte?" Blood 80, 1865-1867 (1992)]. For this reason it is desirable that leukocytes be reduced to the lowest feasible levels.

It would therefore be highly desirable to have a method for removing leukocytes along with other debris from whole blood or blood fractions quickly and efficiently.

In addition to the removal of leukocytes, there is also a need to remove viral inactivating agents. In recent years there has been great interest in inactivating viruses such as Hepatitis B (HBV), Hepatitis C (HCV), Human T Lymphotrophic Retrovirus Type 3 (HTLV), Human Immunodeficiency Virus (HIV), and Lymphadenopathy Associated Virus (LAV) in blood and blood products. At present, methods for inactivating these viruses in blood and blood fractions include (1) treatment with a chemical disinfectant such as formaldehyde (see US patent 4,833,165); and (2) treatment with photosensitizers. For example, U.S. Patent 5,232,844 describes the use of phthalocyanines; U.S. Patent 5,041,078 describes the use of sapphyrins; U.S. Patents 4,169,204, 4,294,822, 4,328,239 and 4,727,027 describe the use of various furocoumarins (psoralens) and analogs thereof; Meruelo et al. [Proc. Nat. Acad. Sci. U. S., 85, 5230-5234 (1988)] have described the use of hypericin; Lambrecht et al. [Vox Sang. 60, 207-213 (1991)] have described the use of phenothiazine dyes (methylene blue and toluidine blue); and U.S. Patent 4,915,683 describes the use of merocyanine dyes to inactivate viruses. According to these methods, an exogenous photosensitizer is added to the blood or blood fraction and the solution is irradiated with light of appropriate wavelengths to inactivate the virus.

For example, methylene blue has been added to plasma intended for transfusion. Although methylene

blue exhibits effective virucidal activity and is considered generally safe, it nevertheless represents an exogenous component in the plasma with possible long-term adverse effects not yet fully understood. Other viral inactivation agents such as psoralens carry similar risks.

In all of the foregoing treatments, exogenous agents are added to the biological fluid. In most cases, these exogenous agents must be removed from the biological fluid before it can be administered to a human.

The present invention offers a method for removing antiviral agents after their virucidal function is completed.

European application 239,859 describes a method that is currently employed to remove lipid soluble process chemicals from biological fluids. It comprises bringing the fluid into contact with a naturally occurring oil, agitating the resultant mixture, separating the phases by sedimentation or centrifugation, decanting the upper lipid phase, and utilizing the residual biological fluid. Aside from the mechanical complexity of this process, it appears applicable only to the removal of lipid soluble process chemicals.

Gel filtration is also known for removing small molecules from blood fractions based on molecular weight differences. Horowitz et al. [*Transfusion, 25, p. 516-522 (1985)*] have described the removal of tri-n-butyl phosphate from anti-hemophilic factor concentrates by chromatography on Sephadex G-25; however, gel chromatography is not a practical method

for removing small molecules from plasma and whole blood.

PCT application WO 91/03933 discusses the use of silica gel, modified silica gel, glass beads, and amberlite resins to adsorb methylene blue from plasma. None of the methods presently in use or proposed is particularly attractive for the routine processing of plasma.

Media and devices for removing leukocytes from red blood cell concentrates, platelet concentrates, and other blood fractions have been described. The media are typically non-woven mats of controlled fiber diameter that rely in large part on mechanical entrapment of cells.

Pall and Gsell (U.S. patent 4,880,548) disclose a device for the depletion of leukocyte content in a platelet concentrate. The device incorporates a modified porous, fibrous medium in which the critical wetting surface tension has been elevated to about 90 dynes/cm by chemically attaching to the fiber surface a high density of hydroxyl groups. This is done by grafting hydroxyethyl methacrylate. Pall (U.S. patent 4,925,572) extends this concept to a device incorporating three elements, the third of which, for removing leukocytes, has been modified to a critical wetting surface tension of 53 to 90 dynes/cm by coating with polymers such as mixtures of 2-hydroxyethyl methacrylate and methylmethacrylate or by chemical reaction to modify the substrate polymer such as by oxidation. Nishimura et al. (U.S. patent 4,936,998) takes a similar approach using a filter of polyethylene terephthalate fibers with a surface that is a copolymer of 2-hydroxyethyl methacrylate and

diethylaminoethyl methacrylate.

Beutler, et al [J. Lab. Chem. Med. 88, 328-333 (1976)] describes a method for the removal of leukocytes from whole blood by passing the blood through a bed composed of microcrystalline cellulose and α-cellulose.

A leukodepletion filter element is described in U.S. Patent 5,190,657. Such depth filters are useful as components for fabricating a filter device described below as the second apparatus aspect of the instant invention, and they provide a starting point for adding ligands for leukocytes and viral inactivating agents.

There is a report (Japanese application 02-167071) of a system for isolating non-human animal B lymphocytes by affinity chromatography on a column of various particles of 0.05 to 5 mm diameter having saccharides attached thereto. Such affinity columns are often useful when the object of the process is to obtain a specific cell type, but they are usually of limited utility when the object is to purify blood or a blood fraction because of the risk that other cellular components (e.g. red blood cells) that are needed in the final product may be differentially retarded or damaged. In addition, many substrates suitable for chromatographic media cause hemolysis. Hemolysis is not a problem when obtaining lymphocytes for immunological research is the goal; however, it is a critical problem when obtaining blood or blood fractions for transfusions.

Summary of the Invention

The present invention provides a filter and a method for removing leukocytes, viral inactivating agents, or both simultaneously, from plasma or other blood fractions quickly, safely and with very high efficiency. Typical viral inactivating agents that can be removed include:

- psoralen, psoralen derivatives, and related compounds;
- hypericin, its derivatives, and related compounds;
- virus-inactivating aldehydes including formaldehyde; and
- various dyes (e.g., methylene blue, toluidine blue, crystal violet) and their photodecomposition products.

The present invention permits efficient, integrated (i.e., single-step) removal of both leukocytes and virus-inactivating compounds from plasma -- thus producing a blood product free of active viruses, antiviral compounds, and leukocytes. The invention also provides a method for attaching ligands for viral inactivating agents and ligands for leukocytes to commercially available filters. A particular advantage of the present invention is its ability to effect blood decontamination without subjecting whole blood to chemical and/or flow conditions that would otherwise tend to cause coagulation and red blood cell hemolysis, problems encountered when whole blood is passed through chromatography columns.

In a first method aspect the invention relates to a method for removing leukocytes from blood

products, such as plasma by passing the plasma through a novel filter for removing leukocytes. The novel filter, which constitutes the first apparatus aspect of the invention, comprises a laid textile web which includes a mechanically stable polymeric substrate, at least a portion of the polymeric substrate having covalently attached thereto a ligand. The ligand has an affinity for the leukocyte cell surface and can be attached directly to the substrate or can be attached through an intervening linker or multiple linkers. Suitable ligands include glycoproteins of the selectin family and carbohydrates, particularly sulfoglycans such as heparin. The linker, when present, can be the residue of an alkylene diamine such as ethylene diamine.

A particular embodiment of the filter for removing leukocytes comprises a shape-sustaining laid textile web having a thickness of 1 to 8 mm and a bulk density of 0.05 to 0.4 g/cm^3, said web comprising:

(a) a plurality of interlocked textile fibers with average deniers between 0.05 and 0.75 and average lengths between 3 mm and 15 mm, said textile fibers being substantially uniformly distributed in said web so as to form a matrix of the textile fibers with spaces between adjacent interstices of interlocked fibers; and

(b) a plurality of fibrillated particles of surface-modified polymeric material having a surface area of 5 to 60 square meters per gram substantially disposed within said spaces of the matrix, said fibrillated particles having a plurality of fine fibrils which are interlocked

with adjacent textile fibers of said spaces such that the fibrillated particles are not substantially displaceable from said web during filtration of said blood;

wherein the weight ratio of the fibrillated particles to the textile fibers is between 1:99 and 40:60; and

wherein said surface-modified polymeric material is a hemolytically inert polymer covalently linked to a ligand for leukocytes; and

wherein said textile fibers are hemolytically inert and stable to alkaline hydrolysis.

In a preferred embodiment the surface modified polymeric material is a cellulose ester, most preferably cellulose acetate, the ligand for leukocytes is heparin, and the linker is ethylene diamine. The textile fibers in the filter may be one or more of polyolefin, polyamide, polysulfone, polyester, polyvinyl alcohol and poly(ethylene-vinyl alcohol) copolymer fibers; polyolefin or polyolefin-sheathed fibers are preferred.

In a second method aspect, the present invention relates to a method for simultaneously removing leukocytes and one or more viral inactivating agents from blood products, such as plasma by passing the plasma through a filter adapted for removing leukocytes and antiviral agents. "Adapted for removing leukocytes" means having appropriate geometry and surface chemistry to trap at least a portion of available leukocytes while allowing other blood components of interest to pass. It includes filters of the type described in the preceding

paragraphs.

Removal of antiviral agents in a first embodiment of the second method aspect (i.e. the dual removal of leukocytes and antivirals) is achieved by means of sorption onto activated carbon or media containing activated carbon. In this embodiment leukocytes and one or more viral inactivating agents are simultaneously removed from whole blood or a blood fraction by passing the blood or blood fraction through a filter adapted for removing leukocytes and antiviral agents, said filter comprising (1) a mechanically stable polymeric material capable of retaining leukocytes and (2) activated carbon capable of removing the viral inactivating agent. A portion of the mechanically stable polymeric material may optionally have covalently attached thereto a first ligand, which has affinity for a leukocyte cell surface (i.e. a filter according to the first aspect of the invention). In a preferred method, the blood fraction is plasma. In another preferred method pertaining specifically to plasma, the viral inactivating agent is selected from the group consisting of phenothiazine dyes and photodecomposition products of phenothiazine dyes. Preferably, the viral inactivating agent is selected from the group consisting of methylene blue, toluidine blue, and photo-decomposition products of methylene blue and toluidine blue. The mechanically stable polymeric material that retains leukocytes may be included within a laid textile web.

In a second apparatus aspect, a filter device is provided to accomplish the first embodiment of the second method. The device comprises (1) a housing, enclosing (2) an activated carbon-containing

filter element and (3) at least one filter element adapted for retaining leukocytes. The filter element for retaining leukocytes may comprise a laid textile web which may optionally include a mechanically stable polymeric material having attached thereto a first ligand, which has affinity for the leukocyte cell surface. The first ligand may be attached directly to the polymeric material, or it may be attached to the polymeric material through at least one intervening linker. When present, a preferred ligand is heparin.

In a specific embodiment, the foregoing filter device comprises (1) an activated carbon-containing filter element, preferably a carbon/cellulose composite, and (2) a shape-sustaining laid textile web having a thickness of 1 to 8 mm and a bulk density of 0.05 to 0.4 g/cm^3. The web is made up of:

(a) a plurality of interlocked textile fibers with average deniers between 0.05 and 0.75 and average lengths between 3 mm and 15 mm. The textile fibers are substantially uniformly distributed in the web so as to form a matrix of the textile fibers with spaces between adjacent interstices of interlocked fibers; and

(b) a mechanically stable polymeric material comprising a plurality of fibrillated particles of polymeric material having a surface area of 5 to 60 square meters per gram substantially disposed within the spaces of the matrix. The fibrillated particles have a plurality of fine fibrils which are interlocked with adjacent textile fibers of the spaces such that the fibrillated particles are not substantially displaceable from the web during filtration.

The weight ratio of the fibrillated particles to the textile fibers is between 1:99 and 40:60. A preferred polymeric material is cellulose acetate and preferred textile fibers are polyolefin and polyester fibers.

Removal of antiviral agents in a second embodiment of the second method aspect is achieved by means of passage through a filter comprising a mechanically stable substrate which has surface chemistry adapted for removing both leukocytes and viral inactivating agents. The third and fourth apparatus aspects of the invention relate to devices for practicing this second embodiment of the second method aspect.

In a third apparatus aspect the invention relates to a (non-membrane) filter for removing leukocytes and one or more viral inactivating agents from whole blood or a blood fraction. Usually, to remove the viral inactivating agents, it will be necessary to modify the substrate such that at least a portion of the mechanically stable substrate has covalently attached a first ligand, which has affinity for a viral inactivating agent; however, in the case of certain viral inactivating agents, the substrate may itself have sufficient affinity for that specific antiviral to effect adsorption without the need to add specific ligands for viral inactivating agents. The substrate additionally includes a covalently attached second ligand having affinity for the leukocyte cell surface.

In this third apparatus aspect, the filter comprises a laid textile web which includes a mechanically stable polymeric substrate. At least a

portion of the polymeric substrate has covalently attached thereto a first ligand, which has affinity for viral inactivating agents. In one embodiment, the first ligand is attached directly to the polymeric substrate; in another, the first ligand is attached to the polymeric substrate through at least one intervening linker.

The first ligand may be a hydrazine-terminated moiety, a mimic of a viral DNA or RNA, in particular, a polythymidine, a carbohydrate, or a mimic of a viral protein.

The polymeric substrate may have additionally covalently attached thereto a second ligand, which has affinity for the leukocyte cell surface. The second ligand may be a glycoprotein of the selectin family, or a carbohydrate, particularly a sulfoglycan that includes residues of glucuronic acid, such as a heparin. As before, the ligand may be attached directly to the polymeric substrate, or the ligand may be attached to the polymeric substrate through at least one intervening linker. The intervening linker may be the residue of an alkylene diamine, in which case, the linker may be attached to the ligand by an amide bond to a carboxyl of the ligand, when one is present.

A preferred filter is as described above for the method. It comprises a shape-sustaining laid textile web having a thickness of 1 to 8 mm and a bulk density of 0.05 to 0.4 g/cm^3. The web comprises:

(a) a plurality of interlocked textile fibers with average deniers between 0.05 and 0.75 and average lengths between 3 mm and 15 mm, said textile fibers being substantially

uniformly distributed in said web so as to form a matrix of the textile fibers with spaces between adjacent interstices of interlocked fibers; and

(b) a plurality of fibrillated particles of surface-modified polymeric material having a surface area of 5 to 60 square meters per gram substantially disposed within said spaces of the matrix, said fibrillated particles having a plurality of fine fibrils which are interlocked with adjacent textile fibers of said spaces such that the fibrillated particles are not substantially displaceable from said web during filtration of said blood;

wherein the weight ratio of the fibrillated particles to the textile fibers is between 1:99 and 40:60; and wherein the surface-modified polymeric material is a hemolytically inert polymer covalently linked to a plurality of ligands for viral inactivating agents and to a plurality of ligands for leukocytes; and wherein the textile fibers are hemolytically inert. In various embodiments the surface-modified polymeric material is cellulose acetate, and the textile fibers are one or more of polyolefin, polyamide, polysulfone, polyester, polyvinyl alcohol, and poly(ethylene-vinyl alcohol) copolymer fibers. Polyolefin fibers are preferred.

In a fourth apparatus aspect, the invention relates to a hollow-fiber or flat-sheet membrane appropriate for carrying out the second embodiment of the second method aspect of the invention, i.e. for removing leukocytes and viral inactivating agents from a blood fraction or whole blood. The membrane comprises:

(a) polyethersulfone (PES) as the primary

hydrophobic polymer component, said PES having functionalizable phenolic chain ends;

(b) a first linker moiety attached to a plurality of said phenolic chain ends, said first linker derived from an oxirane selected from the group consisting of ethylene glycol diglycidyl ether, 1,4-butanediol diglycidyl ether and epichlorohydrin; and

(c) a surface-modified polymeric material attached to a plurality of said first linker moieties, said surface-modified polymeric material consisting of a hemolytically inert polymer covalently attached to a plurality of ligands for viral inactivating agents and for leukocytes. Preferably, the surface-modified polymeric material is a hydroxyalkylcellulose or polyethyleneimine covalently attached to the ligands through a second linker moiety. Preferred ligands include heparin and polythymidine.

In a third method aspect, the invention relates to a method for producing a leukocyte and/or viral inactivating agent filter appropriate for use in either the first method or the second embodiment of the second method. The method for producing a filter is fundamentally similar in both instances and differs only in the final step of attaching the appropriate ligands for either leukocytes, viral inactivating agents or both. The fabrication method (i.e. the third method aspect of the invention) comprises:

(a) providing a shape-sustaining web comprising:

(1) a plurality of fibers that are resistant to alkaline degradation; and

(2) a plurality of cellulose acetate

fibers;

(b) treating said web with aqueous base to hydrolyze a portion of cellulose acetate esters to the corresponding free hydroxyls;

(c) activating said free hydroxyls to produce amine-reactive residues;

(d) reacting said amine-reactive residues with a diamine or a dihydrazide; and

(e) reacting the resulting amine or hydrazide with an activated derivative of a ligand for a viral inactivating agent, for leukocytes, or for both.

When the viral inactivating agent is formaldehyde or another aldehyde, the final step (e) may be omitted and the terminal hydrazide may be used to sequester the aldehyde.

A specific embodiment of the third method aspect entails:

(a) providing a shape-sustaining laid textile web having a thickness of 1 to 8 mm and a bulk density of 0.05 to 0.4 g/cm^3, said web comprising:

(1) a plurality of interlocked polyolefin fibers with average deniers between 0.05 and 0.75 and average lengths between 3 mm and 15 mm, said polyolefin fibers being substantially uniformly distributed in said web so as to form a matrix of the polyolefin fibers with spaces between adjacent interstices of interlocked fibers; and

(2) a plurality of fibrillated cellulose acetate particles having a surface area of 5 to 60 square meters per gram substantially disposed within said

spaces of the matrix, said fibrillated particles having a plurality of fine fibrils which are interlocked with adjacent polyolefin fibers of said spaces such that the fibrillated particles are not substantially displaceable from said web during filtration of blood, wherein the weight ratio of the fibrillated particles to the polyolefin fibers is between 1:99 and 40:60; and

(b) treating said web with aqueous base to hydrolyze a portion of cellulose acetate esters to the corresponding free hydroxyls;

(c) activating said free hydroxyls to produce amine-reactive residues;

(d) reacting said amine-reactive residues with a diamine or a dihydrazide; and

(e) reacting the resulting amine or hydrazide with an activated derivative of polythymidine or of heparin or a combination of the two simultaneously or sequentially.

The web may be treated with 1 N aqueous sodium or potassium hydroxide at 20 to 40°C for 8 to 312 hours. Variations of the parameters such as the concentration of the sodium hydroxide solution, the nature of the base (e.g. potassium hydroxide), the temperature and time can be used to provide the necessary modification of the polymeric material.

The free hydroxyls may be activated by reaction with an activating agent selected from the group consisting of: cyanogen bromide, carbonyl diimidazole, divinyl sulfone, azlactones, sulfonyl chlorides, diepoxides, dihalides, haloepoxides, 2,4,6-trichloro-S-triazine, 2-fluoro-1-methylpyridium salts, disulfonyl chlorides, periodate, diacid

chlorides, diisocyanates, and haloacetic acid followed by N-hydroxysuccinimide plus a carbodiimide.

In a preferred method, the amine-reactive residues are reacted with adipic dihydrazide to produce a plurality of N-monosubstituted hydrazides.

Brief Description of the Drawings

Fig. 1 is a perspective view of a filter according to the first apparatus aspect of the invention in a configuration useful for placing in a filter carrier.

Fig. 2 is a diagrammatic partial cross-sectional view of a portion of the filter of Fig. 1, taken along line I-I.

Fig 3. is a schematic representation of a filter according to the first apparatus aspect of the invention showing attached ligands for leukocyte surface features.

Fig 4. is a schematic representation of a filter according to the third apparatus aspect of the invention additionally showing attached ligands for viral inactivating agents.

Fig. 5 is a diagrammatic cross-sectional view of a filter device according to the second apparatus aspect of the invention.

Detailed Description Inclusive of Preferred Embodiments

The present invention provides, in one embodiment appropriate to the first apparatus aspect, a filter for simultaneously removing leukocytes and any other debris or components larger than about 8 μm in cross-section, and, in another embodiment, a hollow-fiber or flat-sheet membrane for removing primarily leukocytes, although it could be used for mechanical filtration based on particle size as well, if the membrane and apparatus were appropriately designed. The membrane could also, by proper modification, be used to remove both leukocytes and a portion of the aqueous solution in which the leukocytes are found.

The filter is a modification of the filter described in U.S. patent 5,190,657. The figures from that patent are used in the following description for the sake of clarity. Briefly, the filter consists of a filter material which is a shape-sustaining laid textile web. As shown in FIG. 1, the web has been cut in a circular configuration to form the filter and is suitable for loading into a cylindrical filter carrier.

The thickness of the web is at least 1 millimeter, most preferably at least 2 millimeters, and can be up to about 8 mm. The density of the laid web is between about 0.05 and 0.4 g/cm^3.

As seen in FIG. 2, which is a highly diagrammatic illustration of a portion of a section of the filter of FIG. 1, the filter material is comprised of a plurality of matrix textile fibers 5,

and these textile fibers have average deniers between about 0.05 and 0.75. At least 60%, preferably at least 70% and more preferably at least 80 to 85% of the fibers have deniers within the above-noted ranges, and lengths from 12,000 to 180,000 m/g.

As can be seen in FIG. 2, the textile fibers are substantially uniformly distributed through the web so as to form a matrix of the textile fibers. The matrix has spaces **7** between adjacent interstices **6** of the interlocked fibers. Within these spaces, there are a plurality of fibrillated particles **10** of very high surface area. The fibrillated particles **10** are disposed within spaces **7**, as well as along and among the matrix textile fibers **5**.

A portion of the matrix textile fibers **5** may have a sheath and a core with the deniers and lengths thereof being the same as described above. The sheath is of a low melt temperature polymer, and the core of a higher melt temperature polymer. Low melt temperature polymers generally include polymers with melt temperature below about 190° C. When at least a portion of the matrix textile fibers of the filter material are the sheath/core fibers, the web of the filter material will have been subjected to temperatures such as to soften the polymer sheath and cause some bonding of the textile fibers together. Generally, from 5 to 35% of the matrix textile fibers are the sheath/core.

The matrix textile fibers are commonly synthetic polymer fibers, such as polyolefin or polyolefin-sheathed fibers, polyamide, polysulfone, polyester, polyvinyl alcohol and poly(ethylene-vinyl alcohol) copolymer fibers. Polyolefin fibers are quite

resistant to alkaline hydrolysis and are therefore much to be preferred. When the filter is to be used to separate leukocytes from red blood cells, a critical feature of both the textile fibers and the fibrils is that they be compatible with blood, and, in particular, that they not cause significant hemolysis. In a clinical situation, hemolysis that gives rise to more than 10% increase in free hemoglobin would be considered significant.

The sheath fiber, if used, has a core of the aforementioned textile fiber materials and a sheath of any low melting polymer. Polyolefin polymers, such as polyethylene or polypropylene, are preferred, since they provide sheaths with relatively low melting points, and it is easy to soften the sheaths to provide the required adherence. In addition, they are resistant to alkaline hydrolysis. The sheath will commonly be 5-30% of the core diameter.

The fibrillated particles are polyester fiber material, acrylic fiber material, nylon fiber material, polyolefin fiber material or cellulosic fiber material. Cellulose acetate is usually used since a great number of fibrils are produced with that material, and the material has a natural hydrophilic nature.

The filters used in the examples that follow are commercially available from Lydall Inc. (Manchester, CT) and consist of polypropylene fibers and cellulose acetate "fibrets." In general, any filter comprised of a cellulose acetate component and a shape-sustaining web that is resistant to base hydrolysis is well suited to modification according to the third

method aspect of the invention.

Lydall polypropylene/cellulose acetate filter pads (1.9mm thick, style 825B) are modified by immersing 90 mm diameter discs in aqueous alkaline solutions for varying lengths of time. The pads are washed extensively with water on a filter until the washings are close to neutral, and then dried in air at 30° to 40° C. This hydrolysis converts the cellulose acetate to cellulose containing free hydroxyl groups. Insufficient hydrolysis results in a very low number of hydroxyl groups available for subsequent reaction and hence a low capacity for leukocytes in the resulting filter, whereas extensive hydrolysis results in the degradation of the cellulose substrate. In a particular example, the web is hydrolyzed with aqueous sodium hydroxide. Those skilled in the art will recognize that various temperatures, concentrations of sodium hydroxide and treatment times will affect the extent of hydrolysis and the degradation of the substrate. Generally, treatment conditions comprising sodium hydroxide concentrations higher than 0.1N but lower than 5N at temperatures lower than 60° C, and treatment times of about 8 hours to 13 days provide adequate hydrolysis without excessive degradation of the substrate. For these particular pads, it has been found that treatment with 1N sodium hydroxide at room temperature for more than 8 hours and up to 312 hours provides adequate hydrolysis and acceptable degradation of the substrate. It is expected that other bases, such as potassium hydroxide, lithium hydroxide, etc. would function similarly.

The "hydrolyzed pads", i.e. having free cellulose OH functionalities, can be activated by any

of the commonly known methods. See Immobilized Affinity Ligand Techniques, Greg T. Hermanson, A. Krisna Mallia, and Paul K. Smith Academic Press, Inc., San Diego CA, (1992), p. 51-132, and Affinity Chromatography, A practical Approach, Edited by P.D.G. Dean, W.S. Johnson and F.A. Middle, p. 31-59, IRL Press Ltd. Eynsham, Oxford OX81JJ, England (1987) and U.S. patent 3,389,142, the disclosures of which are incorporated herein by reference. Two preferred methods include cyanogen bromide activation and periodate activation.

Cyanogen bromide reacts with vicinal diols of cellulose to provide imidocarbonate and/or cyanate intermediates. These are highly activated toward nucleophilic attack and can be subsequently reacted with linkers or ligands containing primary amines. The result of the reaction is a ligand or linker covalently attached to the cellulose through a carbamate. The activation reaction is carried out as described by Axen et al. [Nature 214, 1302-1304 (1967)] and Cuatrecasas et al. [Proc. Nat. Acad. Sci. US 61, 636-643 (1968)] or minor modifications thereof.

Periodate activation involves the periodate-induced oxidative cleavage of vicinal diols to aldehydes, which are similarly reactive toward primary amines in the linker or ligand. A reduction step with sodium cyanoborohydride or similar reducing agent is commonly employed to convert the somewhat hydrolytically labile Schiff base to an alkylamine. These reactions are well known to persons of skill in the art.

In the case where a ligand contains reagent-accessible primary amine groups that are not required for its interaction with the surface of a leukocyte, it can, in principle, be attached directly to the activated cellulose as described above. However, in most cases an amine-containing linker will be employed to provide a bridge between the cellulose and the ligand.

When a membrane is to be prepared for the fourth apparatus aspect of the invention, rather than a depth filter, as in the first through third apparatus aspects, multiple layers of linkers will commonly be employed. In that case, the "surface-modified polymeric material" will include a layer of polymer having functionalities for altering the surface properties of the substrate and/or for multiplying the possible points of attachment. For example, a layer of hydroxyethylcellulose (HEC) can be applied to a polysulfone membrane substrate and the ligand for leukocytes can be attached to the HEC via a glycidyl ether and an alkylenediamine. For the purposes of the present invention all of the layers (HEC, glycidyl ether and alkylenediamine) can be considered "linkers" for covalently attaching the ligand to the substrate, although, usually the HEC in this example would be called a coating polymer for the substrate polysulfone.

There are several reasons for employing at least one linker: (1) the ligand may not have a useable functional group for direct attachment to the desired substrate, (e.g. heparin to cellulose acetate); (2) the chemistry to provide stable covalent bonds may be more readily carried out on the linker; and (3) it may be desirable to provide the ligand with some

degree of mobility to allow it better access to the binding site on its target molecule (leukocytes); in this respect the linker functions as a sort of tether between the ligand and the relatively rigid polymer (cellulose) backbone. In most of the examples that follow, ethylenediamine has been employed as the linker between the cellulose matrix and the carbohydrate ligand, but any alkylenediamine (e.g. hexamethylenediamine) could be used in this setting, and other linkers may be considered for other chemistries. In general, any difunctional molecule that can be attached at one of its termini to substrate and at the other to ligand will function in the invention, as long as the chemistry required for covalent attachment doesn't destroy the binding between the ligand and leukocytes.

Selective binding as used herein refers to specific recognition by one molecule (typically referred to as a receptor) of another molecule (typically referred to as a ligand) by the spatial or polar organization of a determinant site on the second molecule. Selective binding between the two molecules occurs where affinity is sufficiently strong. Binding affinity is typically represented by the affinity constant (K_a) for equilibrium concentrations of associated and disassociated configurations, i.e., K_a = [R-L]/[R][L] where [R], [L], and [R-L] are the concentrations at equilibrium of the receptor (R), ligand (L) and receptor-ligand complex (R-L), respectively.

The specific binding interactions of the receptor and ligand molecules typically include reversible noncovalent associations.

It is known that leukocytes specifically bind to endothelial cells and platelets via an interaction between a sialylated, fucosylated lactosamine residue on the leukocyte and a cell receptor on the endothelial cell. The cell receptor is in some cases a glycoprotein of the E-selectin family. (These glycoproteins are often referred to as endothelial leukocyte adhesion molecules or ELAMs in older literature.) In other cases, binding has been shown between a sialyl residue (found on the surface of leukocytes) and other glycoproteins such as the peripheral lymph node homing receptor (also known as "murine Mel 14 antigen", and "the Leu 8 antigen") and P-selectin (granule membrane protein 140, GMP-140, also known as "PADGEM").

The term "selectin" has been suggested for the general class of receptors, which includes ELAM-1 (E-selectin) and GMP-140 (P-selectin), because of their lectin-like domain and the selective nature of their adhesive functions. Another member of the selectin class is the MEL-14 antigen, and its human analog LAM-1, which are cell surface receptors of lymphocytes.

The structure and function of selectin receptors has been elucidated by cloning and expression of full length DNA encoding each of the above receptors. The extracellular portion of selectins can be divided into three segments based on homologies to previously described proteins. The N-terminal region (about 120 amino acids) is related to the C-type mammalian lectin protein family. Since selectin receptors comprise a lectin-like domain, the specificity of the molecules is likely to be based on protein-carbohydrate interactions. Evidence indicates that a

sialylated, fucosylated N-acetyllactosamine unit of the Lewis X antigen, designated as SLX, is a moiety recognized by the lectin region of the selectin receptor. In particular, the evidence shows recognition of this moiety by both E-selectin and P-selectin. Thus, E-selectin, P-selectin or their respective N-terminal fragments are therefore particularly attractive as ligands, although their present expense does not recommend them for commercial implementation.

Of greater present commercial interest is the use of heparin and similar sulfonated polyglucuronic acids to selectively bind leukocytes. The effect seems to be similar to the interaction described above but is presumably mediated through a different surface feature of the leukocyte. This binding phenomenon was first observed by phase contrast microscopy when we noticed that leukocytes appeared to adhere to HyperD™ beads (Sepracor, Inc., Marlborough, MA) that had been coated with heparin (for use in studies not related to leukocyte removal). Further studies indicated that control HyperD™ beads and control agarose beads did not bind leukocytes. Commercial heparin-agarose beads also did not bind leukocytes. This turned out to be a critical observation, and is now believed to be due to the fact that in the commercial process, the chemistry for attaching the heparin to the agarose interfered with the binding. (See below.) Thus, in light of the results with the immobilized heparin of the art, it was particularly surprising to find that heparin could be used for binding leukocytes.

In the case of heparin, chondroitin sulfate and similar glycans bearing carboxylic acid residues, the

carboxylic acid may be activated for reaction with a nucleophile in the linker or substrate. Usually the nucleophilic residue is a primary amine and the activation utilizes any of the procedures well known in the art for forming amide bonds. We have found that EEDQ and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) are particularly useful. Oxidation of the hexose residues of heparin with periodate, as described for cellulose above, gives rise to conjugates that do not appear to bind leukocytes.

The foregoing chemistry, which was applied to the creation of filters for the removal of leukocytes, can also be applied to the creation of hollow-fiber or flat-sheet membranes, which would be considered examples of the fourth apparatus aspect of the invention. In this case, hollow-fiber membranes are prepared according to the methods of PCT published application 90/04609. The resulting derivatized hollow-fibers are then treated as above to attach the ligand.

The second method and apparatus aspects of the invention relate to the removal of viral inactivating agents, among which phenothiazine dyes are of particular interest. Phenothiazine dyes are photochemicals that bind to nucleic acids. Under suitable activation conditions such as long-wavelength UV irradiation, phenothiazine dyes crosslink the DNA and RNA strands in viruses, thereby disabling uncoiling and replication. They also react with membrane structures and they induce the production of virucidal oxygen radicals from molecular oxygen. These characteristics of phenothiazine dyes form the basis of viral

inactivation and certain photochemotherapies. [See PCT application WO 91/03933.] However, the slight excess of phenothiazine dyes used to ensure thorough interaction with viruses and the consequent residue left in the plasma represents some risk to the patient upon transfusion. For example, methylene blue has been suggested to possess a certain level of mutagenicity and other adverse effects may become of concern with long-term exposure associated with regular transfusion. It is therefore desirable to remove the unreacted phenothiazine dyes or their metabolites and photodegradation products from the plasma after the viral inactivation treatment.

In all of the antiviral treatments, exogenous agents are added to the biological fluid. In most cases, these exogenous agents must be removed from the biological fluid before it can be administered to a human. The present invention entails the perfusion of the biological fluid through an appropriately sized filter, which captures both leukocytes and viral inactivating agents. In some embodiments the filter may be designed to enhance the removal of leukocytes through the use of a matrix which is surface treated with carbohydrate-based ligands.

In the case of phenothiazine dyes used as the viral inactivating agents, single donor units of plasma are individually injected with precisely measured amounts of the dye, and mixed thoroughly inside the blood bag. The entire blood bag is then irradiated with fluorescent light or narrow-band red light from light emitting diodes for a prescribed period of time. This practice is fundamentally different from the batchwise treatment of pooled plasma, i.e. large volumes of plasma obtained by

combining many single-donor units. Pooling is convenient from a processing scale viewpoint, but has the disadvantage that a single infected unit of plasma, ie. one carrying pathogens, is capable of contaminating an entire plasma pool. Single-donor unit processing avoids this risk; the practice is also particularly suited for subsequent viral inactivating agent removal with an individual, disposable filtration device to result in a higher quality, individually identifiable unit of plasma.

A filtration device may be sized according to the quantity of treated plasma requiring methylene blue removal. Preferably, the device is designed to remove essentially all of the viral inactivation agent used to treat a single unit of plasma--a highly desirable practice rendered feasible by the methylene blue technique, for example.

In this invention, plasma that has been viral inactivated with methylene blue is brought into contact with a filter medium containing activated carbon in a flow-through device. The activated carbon may be in the form of a discrete sorption layer of powder, granules, fibers, or fabric (woven, knitted, or nonwoven). Alternatively, carbon fibers (filaments or staples) may be incorporated into a filter matrix as one of its components. Another medium may be a porous solid comprising activated carbon as its active ingredient. Yet another medium may be a composite structure, combining one or more forms of activated carbon with other non-carbonaceous structural elements, to provide filtration media with specific sorption, permeability and mechanical properties. In all cases, sorption of methylene blue will take place primarily on the activated carbon

surfaces.

In the flow path of the filter device, the activated carbon medium may be preceded by a depth filter with the capability of removing lipids and solid impurities which may be present. Alternatively, the activated carbon medium itself may be constructed so as to impart lipid and solid retention properties. Another filter may optionally be placed downstream of the activated carbon media to retain fragments or particles that may be released from any of the filter components.

A hydrophilic coating may optionally be applied to the activated carbon surfaces. This coating serves one or more of the following functions: 1) to encapsulate and contain the carbon material, thus reducing release of fine particulates into the filtered plasma; 2) to reduce undesirable interaction between the activated carbon and plasma components by offering a biocompatible surface in direct contact with the plasma; and/or 3) to reduce or prevent sorption of species substantially larger than methylene blue by means of size exclusion, viz. allowing relatively unimpeded permeation of methylene blue and photolytic products compared to that of larger molecules. The "cutoff" molecular weight of the species to be excluded may be controlled by varying the composition of the encapsulating layer. This is a method of reducing binding of desirable proteinaceous components in the plasma, such as coagulation factors, by the activated carbon. Similar considerations apply to the removal of viral inactivation agents other than phenothiazine dyes.

Plasma samples, especially those collected as single-donor units, exhibit a range of properties, the most readily noticeable of which is the presence of chylomicrons. Chylomicrons include a range of lipid species of different sizes and degree of agglomeration. Units of plasma heavily laden with chylomicrons become more noticeably tinted by methylene blue because of preferential sorption of the dye by the lipid, and are correspondingly more objectionable to the user. In addition, such units are more challenging to filter because they tend to clog the pores of filter media.

To reduce the effect of clogging and ensure filtration of a single-donor unit of plasma can be completed within a reasonable time requires that sufficient frontal area be available in the filter. This influences the design of the filter device in terms of packaging the necessary quantity of sorption media into the most favorable aspect ratio, i.e. the ratio of frontal surface area to volume. The filter media may be shaped as layers of flat sheets, or as hollow fibers or cylinders where the plasma flow would be directed through their annular walls.

Activated carbon media offer the advantage of a high-capacity sorbent, which translates to compact filter devices with small holdup volumes, and thus high recovery of the plasma product. With specific grades of activated carbon and/or by applying surface coatings, selective sorption properties may be created to allow removal and retention of different target components in the treated plasma.

To minimize risks associated with repeated transfusion in long-term therapy, it is prudent to

remove as much methylene blue and its photoreaction byproducts (e.g. Azure B, Azure C) as possible after the viral inactivation step, preferably to levels below detectability (ca. 0.02 μg methylene blue/mL plasma). An example of a suitable medium for this purpose is a carbon composite medium in which activated carbon particles are uniformly dispersed and embedded in a cellulose fibrous matrix.

Much of the methylene blue added to plasma becomes associated with the chylomicron or endogenous lipids. Effective clearance of the dye from the plasma therefore also requires simultaneous removal of this lipid fraction. In addition, effective lipid removal would enable excessively lipemic plasma units previously rejected to be processed for transfusion. A fibrous, porous matrix made of lipophilic (i.e. hydrophobic) materials is appropriate for removing plasma lipids. Since removal is accomplished by adsorption and size exclusion mechanisms, preferred media include those with relatively high surface area-to-volume ratios, morphologies favorable to depth filtration (e.g. decreasing effective pore diameter in the direction of flow through the thickness of the filter), and good biocompatibility to prevent excessive non-specific protein adsorption.

Various coagulation factors may be depleted by non-specific adsorption on the filter media. The consequences vary. For example, loss of Factor VIII is less significant than a comparable loss of Factor V, because the former may be replenished using commercially available plasma fraction preparations, while the latter is not. An ideal filter device should minimize changes in coagulation factor content of the plasma before and after filtration. In

practice, some modest degree of removal of coagulation factors may be tolerated because an excess of such factors is present in the human body, and because the volume of plasma transfused typically represents a small fraction of the total plasma volume in the circulatory system.

Platelet-poor plasma used for transfusion typically has a leukocyte burden of about 10^6 per mL. A 3-log reduction to 10^3 per mL is generally considered adequate for transfusion purposes. With the device and method of the invention, leukodepletion may be performed simultaneously with methylene blue removal after methylene blue treatment. Leukodepletion after methylene blue treatment allows both the plasma-borne and leukocyte-borne viruses to be inactivated simultaneously. Studies have shown that a methylene blue concentration of 0.1 μM is adequate for both purposes.

There is some evidence that platelets may be activated by contact with carbon particles. This problem may be addressed effectively in two ways: by coating the carbon surface with a more biocompatible material as discussed above, or by removing the platelets altogether from the plasma by sorption onto appropriate depth filter media.

The filter of the second apparatus aspect of the invention comprises (1) a mechanically stable polymeric material, which may have a surface chemistry adapted for removing leukocytes, and (2) activated carbon or a medium containing activated carbon for removing viral inactivating agents.

A suitable leukocyte depleting medium for use in the device according to the second apparatus aspect of the invention may comprise a laid textile web which includes a mechanically stable polymeric material. In an improved medium, a portion of the polymeric material may optionally have covalently attached thereto a first ligand, which has affinity for a leukocyte cell surface, as described above for the first aspects of the invention. In one embodiment, the first ligand may be attached directly to the polymeric material; in another, the first ligand is attached to the polymeric material through at least one intervening linker.

The first ligand may be a glycoprotein of the selectin family, or a carbohydrate, particularly a sulfoglycan that includes residues of glucuronic acid, such as a heparin. The intervening linker may be the residue of an alkylene diamine, in which case, the linker may be attached to the ligand by an amide bond to a carboxyl of the ligand, when one is present.

Other ligands now known or subsequently discovered are expected to function similarly. The critical requirements of the leukocyte ligand are a high affinity for the leukocyte surface and a functionalizable substituent at some position remote from the binding region whereby the ligand can be covalently bound to the polymeric material.

In operation, an individual unit of plasma (or if necessary, some larger volume of plasma being processed) that is suspected of being contaminated by a virus will be treated by the addition of an effective concentration of a virus-inactivating

phenothiazine dye to the plasma. The unit of plasma is then irradiated for a sufficient time to permit the antiviral compound to inactivate both "free" (i.e., plasma-borne) and cell-associated virus.

Next, and involving the methods and devices of the present invention, the treated plasma, still containing at least significant amounts of antiviral agent, will be passed through a leukocyte/antiviral filter of the present invention, to produce a plasma product substantially free of active viruses, leukocytes capable of harboring them, and residuals of the antiviral compound itself.

Filters that can remove leukocytes are known in the art. For example, Lydall Inc. manufactures a suitable leuko-depletion filter, and Asahi Chemical manufactures another. More efficient filters activated by attachment of heparin and other ligands capable of enhancing the capture of leukocytes are disclosed below.

Fig. 2 illustrates, in a highly idealized schematic form, one embodiment of a filter element useful in the filter of the present invention. The filter is comprised of a polymeric material **14** to which are covalently bonded a plurality of ligands **15** for leukocyte cell surface features. In use, the leukocytes **11** are held within the filter matrix both by mechanical effects (size) and by specific interactions between binding sites on the ligands **15** and sites on the surface of the leukocytes.

The filter elements used in the examples that follow are commercially available from Lydall Inc. [Hamptonville, NC] and consist of polypropylene

fibers and fibrillated cellulose acetate "fibrets." In general, any filter comprised of a cellulose acetate component and a shape-sustaining web that is resistant to base hydrolysis will function in the fourth method aspect of the invention for preparing surface-modified filters. For the second method and apparatus aspects of the invention, it is desirable, but not necessary, to modify the leukocyte depletion filter.

The particular filter device described below as a preferred embodiment of the second apparatus aspect has the additional feature that it also removes chylomicrons, microaggregates, bacteria and endotoxins from plasma. The combined effects of the various features of the device are quite profound: (a) By removing > 95% of methylene blue and its photolysis products, it eliminates concern about methylene blue toxicity and concern about the visual appearance of the plasma; (b) by removing >99.9% of leukocytes, it improves virus inactivation capability, reduces leukocyte-associated bacteria (e.g. *Yersinia histolytica*), and reduces leukocyte-associated immunologic effects; (c) by removing chylomicrons or lipids it improves the appearance of the plasma, eliminates the need for a microaggregate filter at the bedside, and avoids having to discard highly lipemic plasma units; (d) by removing bacteria, it reduces sepsis; and (e) by removing endotoxins, it reduces or eliminates febrile reactions. All of these advantages are accomplished at low cost, with a plasma volume loss of less than 5%, and the process can be carried out on a single donor unit basis, thereby avoiding the hazards associated with pooling blood supplies.

The second embodiment of the second method aspect of the present invention entails the perfusion of whole blood or a blood fraction (on either a process or single-blood-unit scale) through an appropriately sized leukodepletion filter, the surface of which is additionally made to exhibit an affinity for the adsorptive capture of viral inactivating agents. The surface chemistry of this "leukocyte-depletion/antiviral-capture" filter may either inherently be appropriate to adsorption of the particular antiviral of interest, or the surface may be activated for antiviral capture by attachment of specific ligands or other suitable capture chemistries to remove the potentially toxic antiviral agents, such as psoralens and aldehydes, from red blood cell and platelet concentrates after antiviral pretreatment.

In operation, an individual unit of blood (or alternatively, some larger volume of blood being processed) that is suspected of being contaminated by a virus will be treated by the addition of an effective concentration of a virus-inactivating compound to the blood. Sufficient time will be provided to permit the antiviral compound to inactivate both "free" (i.e., plasma-borne) and cell-associated virus. At this point, a cell washing step may or may not be conducted.

Next, and involving the methods and devices of the present invention, the treated whole blood, still containing at least significant amounts of antiviral agent, will be passed through the leukocyte/antiviral filter of the present invention, to produce a red blood cell product substantially free of active viruses, leukocytes capable of harboring them, and

residuals of the antiviral compound itself.

In its broadest sense, the second method aspect of the present invention is limited neither to a particular morphology or chemistry of the leukocyte filter employed as a substrate, nor to the specific nature of the antiviral compound to be removed, the surface chemistry and/or ligand capable of immobilizing it, or the means of incorporating same into the leukodepletion filter.

The surface chemistry of certain leukocyte filters may effect removal of a substantial fraction of antiviral compounds that may be present, even if no specific ligand capable of binding a target antiviral compound is attached to the filter. In these instances, the inherent surface chemistry of these leukocyte filters will result in adsorption and capture of certain types of antiviral compounds. Their use in integrated processes for the removal of both leukocytes and antiviral compounds is encompassed within the methods of this invention.

Psoralens are photochemicals that bind to nucleic acids. Under suitable activation conditions such as long-wavelength UV irradiation, psoralen crosslinks the DNA and RNA strands in viruses, thereby disabling uncoiling and replication. These characteristics of psoralens form the basis of viral inactivation and certain photochemotherapies. [See Anderson and Voorhees Ann. Rev. Pharmacol. Toxicol. 20, 235-57 (1980).] More recently psoralens have been applied successfully to inactivate blood-born viruses. However, the slight excess of psoralen used to ensure thorough interaction with viruses and the consequent residue left in the blood represents some

risk to the patient upon transfusion -- for example, ocular complications, hyperphotosensitivity, and in some cases carcinogenicity. It is therefore desirable to remove the unreacted psoralen or psoralen derivative from the blood after the viral inactivation treatment, but prior to transfusion. The portion of the psoralen bound to the nucleic acids has no further activity and thus requires no removal.

The interaction mechanism of psoralens with DNA has been extensively studied and reviewed. A preliminary intercalation complex is formed between the psoralen and two base pairs of the duplex DNA via hydrophobic interaction. Subsequent exposure to UV-A irradiation then causes photoconjugation between the furocoumarin structure of the psoralen and one or two bases of the nucleic acid (to form mono- and bi-functional adducts, respectively). Pyrimidine bases, particularly thymine, have been identified as participants in these reactions. Subsequently, the mono-functional adduct may absorb a photon and react with a second base of the complementary strand of the DNA to form a covalent crosslink. [See Anderson and Voorhees *op. cit.* page 240.]

Several approaches to removing residual psoralen derivatives from blood may be considered. Antibodies may be developed with specific affinity toward a unique psoralen derivative. The antibody may then be immobilized onto a substrate (in solid form or as a high-molecular-weight soluble polymer) to provide recognition sites for binding the corresponding psoralen derivative. Subsequent separation of the substrate from the blood then also removes the psoralen. This approach has the advantage of being

highly selective. A disadvantage, however, is the complexity, expense, and uncertainty associated with developing a unique antibody for each psoralen derivative of interest.

The preferred approach, encompassed by the present invention, is to employ a solid substrate whose surface has been designed to mimic the binding sites offered by the virus. By populating the substrate with oligonucleic acids, particularly those containing pyrimidine base groups, such as polythymidine for example, psoralen can be complexed (and could even be photoreacted) via the same mechanism as it would undergo during viral inactivation.

In use, virus-containing blood is first treated with the psoralen derivative of choice, incubated for a sufficient duration for inactivation to take place, then brought into contact with the functionalized solid substrate. At this point only the unbound psoralen remaining in the plasma will be captured on the substrate. A second UV-A irradiation can be optionally applied to activate the 2+2 cycloaddition between the psoralen and pyrimidine residue(s) in the filter matrix.

Various methods may be used to populate the solid substrate surface with ligands. One approach involves coating the substrate with a hydrophilic copolymer, then grafting side chains carrying ligand residues onto the copolymer. The choice of hydrophilic copolymer as host for grafting is predicated by biocompatibility, absence of complement activation, and its ability to accept subsequent reaction with the ligand moiety. Grafting offers the

advantage that the ligand sites may be spaced away from the polymer surface to reduce steric hindrance during scavenging of the psoralen.

In the case where the ligand is to be a polynucleotide, such as poly(thymidine), an unphosphorylated 3'-terminal ribose residue may be activated with cyanogen bromide or periodate as described above for cellulose. A preferred method is the periodate oxidation described below for ATP activation, derived from Lamed et al. [Biochim. Biophys. Acta 304, 231-235 (1973)] which is incorporated herein by reference. The activated ligand is then reacted with the amine functionality of the linker as described herein for reaction of activated cellulose or activated ATP with linker.

In cases where it is desired to use oligopeptide mimics of viral proteins as ligands, the C-terminal carboxylic acid residue of an N-protected oligopeptide may be activated with a carbodiimide and coupled with the amine-terminal linker as described below for reaction with heparin. If desired, the N-terminal protection can then be cleaved by methods well-known in the art.

Other intercalating viral inactivating agents may be immobilized from blood and blood fractions using the same approach. Photoinactivating agents that do not intercalate can be trapped using a similar approach: a structure that mimics the binding of the inactivating agent on the viral surface protein or similar target structure can be covalently attached to the leukocyte filter substrate as discussed below for carbohydrates and nucleic acids. The chemistry necessary for the extension to other

ligands will be readily appreciated by persons of skill in the art.

The examples which follow include some experiments wherein the ligand for leukocytes was attached to an amine-modified methacrylamide gel which was itself supported in a silica matrix (HyperD™ beads). This was done because the testing of the binding with the beads is faster and less expensive than the testing on complete filter devices or membranes. The results of binding between leukocytes and beads appear predictive of results on filters.

Other ligands now known or subsequently discovered are expected to function similarly. The critical requirements of the leukocyte ligand are a high affinity for the leukocyte surface and a functionalizable substituent at some position remote from the binding region whereby the ligand can be covalently bound to the substrate. The critical requirements of the viral inactivating agent ligand are a high affinity for the viral inactivating agent and a functionalizable substituent at some position remote from the binding region whereby the ligand can be covalently bound to the substrate.

When it is desired to produce filters having both leukocyte ligands and viral inactivating agent ligands, the appropriate activated ligands can be added as a mixture to the substrate when they are chemically compatible. Thus a mixture of periodate-activated heparin and periodate-activated poly(thymidine) may be added in the desired proportion for the final ligand ratio. When the two ligands are chemically incompatible or when one may

sterically hinder the addition of another, they may be added to the substrate sequentially, the order and proportions being readily determined by persons of skill in the art.

PREPARATION OF MODIFIED MATRICES FOR BOTH METHOD ASPECTS OF THE INVENTION

A. Preparation of filters with heparin covalently attached.

Polypropylene/cellulose acetate filter pads (1.9mm thick, style 825B, Lydall Westex, P.O. Box 109, Hamptonville, NC 27020) were modified by immersing 90 mm diameter discs in 1N sodium hydroxide solutions for 44 hours at room temperature. The pads were washed extensively with water on a filter until the washings were close to neutral, and then dried in air at 30° C.

1. Cyanogen Bromide (CNBr) Activation

Pads hydrolyzed for at least 8 hours in 1N sodium hydroxide are used as starting material. A standard procedure is used, keeping the ratio of 2 g of CNBr per gram of filter pads (4 g CNBr/90 mm diameter disc). The filter pads are placed in 500 mL Nalgene plastic filter holders, the bottom, porous membranes of which have been removed to improve flow rate. The filter pads are treated with the CNBr by repeated filtration. The CNBr activated pads are then reacted with a saturated solution of ethylenediamine (approximately 90 g/L) in 0.1M sodium carbonate buffer (pH 9.5), and the reaction allowed to proceed overnight at 4° C, as described by Lamed et al.

[Biochim. Biophys. Acta 304, 231-235 (1973)] for agarose beads. The treated pads may be further derivatized as described below.

2. Oxidation Method

Ten 90mm pads were reacted with 1 liter of 0.5M sodium metaperiodate at room temperature for 3 hours on a Nalgene plastic filter, the bottom membrane of which had been removed. The pads were washed extensively with water, and then reacted with 1M ethylenediamine, (Aldrich Chemical Co., Milwaukee, WI) at pH 7.5 for 16 hours. For each liter of diamine solution, approximately 10 pads were treated. At the end of 16 hours, solid sodium cyanoborohydride was added to the solution to bring the concentration to 0.1M in cyanoborohydride, and the reaction was allowed to proceed for 4 hours. Finally the pads were extensively washed with water (to remove the excess ethylenediamine and sodium cyanoborohydride) and dried overnight at 30 C.

Heparin (Kabi Pharmacia, Sodium Salt, Cat No. H0178), 1.68 g was dissolved in 75.6 ml deionized water. EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, Aldrich Chemical Co.) 504 mg was dissolved in 56.8g of 95% ethanol. The heparin and EEDQ solutions were then mixed at room temperature for 30 min. and reacted with seven 90 mm aminated pads for 6 hrs by soaking and continuously passing the solution through the filter on a Nalgene filter holder. The pads were washed with 1 liter of 95%

ethanol to remove the excess EEDQ, and washed with water to remove excess heparin. The pads were capped with 600 mL of 2M sodium acetate containing 22.5 mL of acetic anhydride for 1 hr. The pads were finally washed with a large excess of deionized water and dried for 72 hours at 30 C. The pads were tested in the device for leukocyte removal described below. The same method may be used for the attachment of any ligand that contains a carboxyl group not involved in its interaction with ligate.

A1. Preparation of filters with heparin covalently attached (square pads).

Lydall polypropylene/cellulose acetate filter pads (grade No. 825, Lot No. 3478, Roll No. 2-1-05) were cut into 27 strips 34.3 cm long. Nine each of the strips were then soaked in separate containers containing four liters of 1N sodium hydroxide at room temperature for 8, 16, and 44 hours respectively. The filter pads were removed at the end of the soaking period and placed on top of a square vessel containing polypropylene filter netting. The square vessel is designed to accommodate several layers of filter pads with a port for draining or applying vacuum, and the introduction of washing solution so that the filters can be washed efficiently. The filters were washed extensively using deionized water to remove any excess sodium hydroxide trapped in the filters, and the filters were sucked dry, until the washings were neutral. The filters on the square vessel were then soaked in 3.6 liters of 0.29M sodium periodate and the solution was circulated through the filters for 4 hours, and washed extensively with deionized water to remove the excess periodate. 1M solutions of ethylene diamine (Aldrich Chemicals), pH 7.5, 3.6 liters, were made by mixing with water and

adjusting the pH with concentrated HCl. The solutions were reacted with the oxidized filters for 16 hours. At the end of 16 hours, solid sodium cyanoborohydride, 23.8g (Sigma) was added to the ethylene diamine solution, filtered through a Nalgene 0.2 μm filter to remove particulates, and further reacted for another 6 hours with recirculation. The filters were then washed extensively using deionized water to remove any excess reagents trapped in the filters, and dried overnight at 30° C.

Heparin (Kabi Pharmacia, Sodium Salt, Cat No. H0178) 34.2 g was dissolved in 1.538 liters of deionized water. EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, Aldrich Chemical Co.) 10.3g was dissolved in 1465 mL of 95% ethanol. The heparin and EEDQ solutions were then mixed at room temperature for 30 min, and allowed to soak the dry ethylene diamine filters placed in the square vessel for 30 minutes. The heparin solution was recirculated through the filter for 3.5 hours and soaked for an additional 20 hours. The filters were washed with 2 liters of 95% ethanol to remove the excess EEDQ, 2 liters of 50/50 ethanol water, and finally with a large excess of water to remove unreacted heparin. The unreacted nucleophilic groups on the filter surface were capped with 3 liters of 2M sodium acetate containing 112.5 mL of acetic anhydride for 1 hr. The filters were finally washed with a large excess of deionized water, and dried at 30° C. Filter pads were cut using a die for testing in the device for leukocyte removal. Pieces of the pads were also tested by mixing with plasma containing leukocytes and examined for leukocyte binding to individual fibers.

A2. Preparation of filters with ATP covalently attached.

Polypropylene/cellulose acetate filter pads were modified as described in A.

1. Cyanogen Bromide (CNBr) Activation

Unhydrolyzed pads and pads hydrolyzed for 8 hours in 1N sodium hydroxide were used as starting material. A standard procedure was used as described in A1. The CNBr activated pads were then reacted with a saturated solution of adipic dihydrazide (approximately 90 g/L) in 0.1M sodium carbonate buffer (pH 9.5), and the reaction was allowed to proceed overnight at 4° C, as described by Lamed et al. for agarose beads. The hydrazide-treated pads may be further derivatized as described below, or they can be used, as is, for removing aldehyde viral inactivating agents.

2. Oxidation Method

Ten 90mm pads were reacted with 1 liter of 0.5M sodium metaperiodate at room temperature for 3 hours on a Nalgene plastic filter, the bottom membrane of which had been removed. The pads were washed extensively with water, and then reacted with 2% adipic dihydrazide, (Aldrich Chemical Co., Milwaukee, WI) at pH 7.4 for 4 hours. For each 1 liter of hydrazide solution, approximately 10 pads were treated. At the end of 4 hours, solid sodium cyanoborohydride (0.1 mol) was added to the solution and reacted overnight, and finally the pads were extensively washed with water on the Nalgene filter.

ATP 5.5 g (0.01 M) was dissolved in 1 liter of deionized water at room temperature, and the pH was adjusted to 4.5 using 10 N sodium hydroxide solution. Sodium metaperiodate, 3.2g (0.015 M) was dissolved in 500 mL of water separately in a beaker and the pH was adjusted to 4.5. Care was taken to cover the periodate solution in order to minimize the exposure to light. The contents of the two beakers were mixed well and kept in the dark for 3 hours. The resulting solution of oxidized ATP was added to 12 adipic dihydrazide-treated filter pads and the pads allowed to stand overnight at room temperature. Solid sodium cyanoborohydride 3.14g (0.05M, Sigma) was then added to 1 liter of the ATP solution and reacted for an additional 4 hours with the pads. Finally the pads were extensively washed with water to remove the excess ATP and sodium cyanoborohydride. The method can be used to attach any ligand in which an aldehyde can be generated without destroying the ligand-ligate interactions.

B. <u>Preparation of hollow-fibers with heparin covalently attached.</u>

Polyethersulfone hollow-fibers (thirty-six bundles, each containing 90 hollow fibers of 1500 microns outside diameter, and 1000 micron inside diameter) prepared as described in copending application 07/956432 on page 87 are placed in a container with slots for the individual bundles, and washed with 20 liters of acetonitrile for 16 hours. The container is provided with ports for draining and a pump for recirculating the liquids to the top of

the container, as well as an on-line heater to heat the solutions as needed, and a reservoir for mixing. The acetonitrile is then drained from the container, and the fibers are washed twice for 10 minutes with 20 liters of deionized water.

The fibers are reacted with a solution of 27 liters of deionized water, 3 liters of ethylene glycol diglycidyl ether (EGDGE, Aldrich Chemical Co., Milwaukee, WI) and 240 grams of 50% sodium hydroxide solution. The solution is allowed to circulate through the fibers in the container for 3 hours, drained from the container, and the fibers are washed twice for 10 minutes with 20 liters of deionized water.

The fibers are reacted with a solution of 27 liters of deionized water, 2 kg of 30% polyethyleneimine solution (PEI) (Epomin P-1000, Aceto Corporation, Lake Success, NY) and 1440 grams of 50% sodium hydroxide solution. The solution is allowed to circulate through the fibers in the container for 5 minutes and the on-line heater is then set such that when the solution is circulated, the temperature of the solution in the container reaches 75° C. The solution is circulated for 3 hours, drained from the container, and the fibers are washed twice for 10 minutes with 20 liters of deionized water. The water is drained and 30 liters of fresh deionized water is added to the container and allowed to circulate for 16 hours. The water is drained and replaced with 27 liters of deionized water.

Two hundred forty grams of premixed solid phosphate buffer salts (Sigma Chemical Co., St.

Louis, MO) is added to the reservoir and allowed to mix for 5 minutes to achieve pH 7.4. Three liters of glutaraldehyde solution (25%, Aldrich Chemical Co.) is added to the reservoir and mixed for 5 minutes. The solutions are circulated through the fibers in the container for 4 hours, drained, and rinsed with deionized water 4 times as described previously. A second PEI coating can be applied to the fibers, if desired, by repeating the process.

The water is drained and 16 liters of 1M ethylenediamine adjusted to pH 7.5 with HCl is added and circulated for 16 hours. At the end of 16 hours, 100g of solid sodium cyanoborohydride is added to the reservoir and the solution is circulated through the fibers for 5 hours at room temperature. The contents are drained, and washed 4 times with deionized water as described previously, and dried at 37°C for 16 hours. The fibers are stored at room temperature until used. They may be further derivatized or used as is to remove aldehyde viral inactivating agents.

Ninety amine-treated hollow fibers (40 cm long, 1.5 mm outside diameter and 1 mm inside diameter, approximately 10g dry weight) are cut into 10cm lengths, and inserted into a 1 liter Nalgene plastic flask. One liter of EEDQ-activated heparin is then introduced into the flask and mixed overnight. The solution was drained from the fibers, and the fibers are extensively washed with ethanol, ethanol or water and then water and dried in air.

B1. <u>Preparation of hollow-fibers with ATP covalently attached.</u>

Polyethersulfone hollow-fibers (thirty-six bundles, each containing 90 hollow fibers of

1500 microns outside diameter, and 1000 micron inside diameter) prepared as described in copending application 07/956432 on page 87 were placed in a container with slots for the individual bundles, and washed with 20 liters of acetonitrile for 16 hours. The container was provided with ports for draining and a pump for recirculating the liquids to the top of the container, as well as an on-line heater to heat the solutions as needed, and a reservoir for mixing. The acetonitrile was then drained from the container, and the fibers were washed twice for 10 minutes with 20 liters of deionized water. The fibers were then reacted with a solution of 27 liters of deionized water, 3 liters of ethylene glycol diglycidyl ether (EGDGE, Aldrich Chemical Co., Milwaukee, WI) and 240 grams of 50% sodium hydroxide solution. The solution was allowed to circulate through the fibers in the container for 3 hours, drained from the container, and the fibers were washed twice for 10 minutes with 20 liters of deionized water. The fibers were reacted with a solution of 27 liters of deionized water, 2 kg of 30% polyethyleneimine solution (PEI) (Epomin P-1000, Aceto Corporation, Lake Success, NY) and 1440 grams of 50% sodium hydroxide solution. The solution was allowed to circulate through the fibers in the container for 5 minutes and the on-line heater was then set such that when the solution was circulated, the temperature of the solution in the container reached 75° C. The solution was circulated for 3 hours, drained from the container, and the fibers washed twice for 10 minutes with 20 liters of deionized water. The water was drained and 30 liters of

fresh deionized water was added to the container and allowed to circulate for 16 hours. The water was drained, and replaced with 27 liters of deionized water. Two hundred forty grams of premixed solid phosphate buffer salts (Sigma Chemical Co., St. Louis, MO) was added to the reservoir, and allowed to mix for 5 minutes to achieve pH 7.4. Three liters of glutaraldehyde solution (25%, Aldrich Chemical Co.) was added to the reservoir and mixed for 5 minutes. The solutions were circulated through the fibers in the container for 4 hours, drained, and rinsed with deionized water 4 times as described previously. A second PEI coating was applied to the fibers by using a solution made up of 27 liters of deionized water and 2 kg of PEI solution, and mixing for 5 minutes. The contents were allowed to circulate at room temperature for 2 hours, and the contents drained. The fibers were then washed with 20 liters of deionized water 4 times, with the final wash circulating for 16 hours. The water was drained, and replaced with 27 liters of deionized water. Two hundred forty grams of premixed solid phosphate buffer salts (Sigma Chemical Co., St. Louis, MO) was added to the reservoir, and allowed to mix for 5 minutes to achieve pH 7.4. Three liters of glutaraldehyde solution (25%, Aldrich Chemical Co.) was added to the reservoir and mixed for 5 minutes. The solutions were circulated through the fibers in the container for 4 hours, drained and rinsed with deionized water 4 times as described previously.

The water was drained and 30 liters of deionized water was added to the reservoir. Adipic dihydrazide, 600g(Aceto Corporation), and sodium cyanoborohydride, 100g (Aldrich Chemical Co.) were added to the reservoir and mixed for 5 minutes. The pH of the solution was adjusted to 7.0 using 5% sodium hydroxide and 1:10 diluted hydrochloric acid solution. The solution was circulated through the fibers for 5 hours at room temperature. The contents were drained, and washed 4 times with deionized water as described previously, and dried at 37°C for 16 hours. The fibers were stored at room temperature until used. They may be further derivatized or used as is to remove aldehyde viral inactivating agents.

Ninety hydrazide-treated hollow fibers (40 cm long, 1.5 mm outside diameter and 1 mm inside diameter, approximately 10g dry weight) were cut into 10cm lengths, and inserted into a 1 liter Nalgene plastic flask. One liter of oxidized ATP at pH 4.5 was then introduced into the flask and mixed overnight. The pH of the oxidized ATP solution was adjusted to 7.4, and solid sodium cyanoborohydride, 3.14g (0.05M) was then added to the flask, and mixed for an additional 4 hrs. The solution was drained from the fibers, and the fibers were extensively washed with water and dried in air.

C. Preparation of Beads with Carbohydrates.

The modification of filter pads is inefficient for examining a large number of substrates and chemistries for leukocyte binding. Therefore, a number of carbohydrate ligands to be

tested for binding to leukocytes were coupled to aminated HyperD™ beads (Sepracor, Inc., Marlborough, MA) containing 36 meq of amine groups/mL settled beads using EEDQ chemistry. HyperD™ beads are porous silica beads having a gel composed of crosslinked aminated methacrylamide derivative in the pores, as described in U.S. patent 5268097 which is incorporated herein by reference.

The ligand (20 to 21 mg) was dissolved in 0.9 mL of water for 1 hour at room temperature. The EEDQ (102 mg) was dissolved in 11.17g of 95% ethanol. The beads were suspended in 50/50 ethanol/water in a 15 mL centrifuge tube. Equal volumes of ligand solution and EEDQ solution were mixed for 30 min at room temperature, and then added to 1 mL of the beads and mixed vigorously for a few minutes. The tubes were then placed in a mixer and reacted overnight at room temperature for 18 hours, washed three times with 50/50 ethanol/water, and four times with deionized water, and finally twice with phosphate buffered saline, pH 7.4. The beads were kept at 4° C until used.

D. Immobilization of carbohydrates using different chemistries and beads.

The carbohydrates were also immobilized to different types of beads using different chemistries. The beads, chemistries and ligands immobilized are described below. The starting glass amino beads were prepared as follows: Glass beads (50g, 50-100 micron, Polysciences, Inc., Warrington, PA) were immersed in a 500 mL glass beaker containing 200 mL of 5% (v/v) nitric acid at 80° C for 2 hours. The beads were washed five times with 400 mL of deionized water, and the excess water decanted. The beaker

containing the glass beads was transferred to an oven, and the oven set at 170° C, and dried overnight. A solution was made by mixing 95 mL methyl sulfate (Aldrich), 5 mL of 3-aminopropyltrimethoxy silane, (Aldrich) and 0.5 mL of triethylamine (Aldrich), and added to the cooled beaker containing beads. The beaker was covered, and placed in a water-bath at 90° C for 24 hours. The beads were then washed 6 times each by settling and decantation with methyl sulfoxide, 1-propanol, and water. The beads were stored in water until used.

The EEDQ and EDC chemistries will immobilize the carbohydrate through the carboxylic acid group, whereas periodate will immobilize the carbohydrate through disrupted hexose units. The immobilization of ligands was carried out using 1 mL of settled beads and reacted with the amount of ligand shown in Table I and reagent which had been premixed for 30 minutes as in B. The ligand was dissolved or dispersed in 0.9 mL of water (for EEDQ reactions) or 1.0 mL of 0.1 M 2-(N-morpholino) ethanesulfonic acid buffer (MES) at pH 4.5 (for EDC reactions). Reagent solutions were 0.9 mL of a solution of 102 mg of EEDQ in 11.27g of 95% ethanol or 0.2 mL of a solution of 150 mg of EDC in 10 mL of 0.1M MES buffer at pH 4.5. The reaction was allowed to proceed overnight, and the excess reagents were removed by extensive washing initially with the same solution in which the reaction was carried out, then with water and finally with phosphate-buffered saline (PBS) pH 7.4. The 1 mL of settled beads were then finally suspended in 1 mL of PBS and stored at 4° C until used.

Table I

Example #	Substrate	Ligand	mg of ligand[a]	Coupling Agent
1	HyperD™ amine	heparin (Kabi)	25	EEDQ
2	HyperD™ amine	heparin (Sigma)	25	EEDQ
3	HyperD™ amine	hyaluronic acid	25	EEDQ
4	HyperD™ amine	heparin (Kabi)	25	EDC
5	HyperD™ amine	heparin (Sigma)	21	EDC
6	HyperD™ amine	chondroitin sulfate A	21	EDC
7	HyperD™ amine	chondroitin sulfate B	20	EDC
8	HyperD™ amine	chondrosine	22	EDC
9	HyperD™ amine	alginic acid	23	EDC
10	HyperD™ amine	hyaluronic acid	20	EDC
11	glass-amino	heparin (Kabi)	25	EEDQ
12	glass-amino	heparin (Sigma)	25	EEDQ
13	glass-amino	chondroitin sulfate A	22	EEDQ
14	glass-amino	chondroitin sulfate B	22	EEDQ
15	glass-amino	chrondrosine	20	EEDQ
16	glass-amino	hyaluronic acid	21	EEDQ
17	glass-amino	heparin (Kabi)	26	EDC
18	glass-amino	heparin (Sigma)	25	EDC
19	glass-amino	chondroitin sulfate A	23	EDC
20	glass-amino	chondroitin sulfate B	23	EDC
21	glass-amino	chondrosine	21	EDC
22	glass-amino	alginic acid	24	EDC
23	glass-amino	hyaluronic acid	21	EDC

[a] in 10 mL of MES buffer (EDC) or 11.27g of 95% ethanol (EEDQ)

TESTING OF MODIFIED MATRICES

The derivatized beads of Preparation C and D were examined under the phase contrast microscope

(Zeiss) using an objective of 40x magnification and an eye piece of 10x magnification. In this case approximately 0.25 mL of beads were mixed with 2mL of blood plasma containing leukocytes for 30 minutes with gentle mixing. Samples were removed and examined under the microscope for attachment of leukocytes to beads. The spherical shape of the beads allowed only one hemisphere of each bead to be observed for leukocyte binding. From each sample, four representative spherical beads were selected and examined for the number of leukocytes bound. The total number of leukocytes observed to bind to the top half of the four beads is given as the total. The results are also given in Table II. It should be noted that only the top half of the beads were examined for leukocyte binding, and the total binding per bead would be expected to be twice the number observed. It was found that heparin derivatized beads bound leukocytes more than the other ligands. In addition, commercial heparin beads were also examined for leukocyte binding but none was observed. Only Heparin HyperD™ bound any leukocytes.

Table II

Substrate	Ligand	Leukocytes observed per 1/2 bead			
		#1	#2	#3	#4
Type I agarose	Heparin	1	0	0	0
Type II agarose	Heparin	0	0	0	0
HyperD™ beads	Heparin	4	3	6	3
Sepharose® C1-4B	None (control)	0	0	0	0
HyperD™ F beads	None	0	0	0	0
HyperD™ F beads	Heparin	2	0	0	0
HyperD™ F beads	Chondroitin sulfate A	0	0	0	0
HyperD™ F beads	Chondroitin sulfate B	0	0	0	0
HyperD™ F beads	Chondrosine	0	0	0	0

The results shown in the preliminary experiments summarized in Table II indicated that under certain circumstances leukocytes adhered to heparin-coated substrates. It was subsequently determined that the binding of heparin to substrate had to be through a carboxylic acid residue if the resulting material is to exhibit leukocyte binding. With that in mind, the experiments described in Preparation C were undertaken. The variations in chemistry, substrate and ligand and their effects on binding are shown in Table III. In the standard experiments 0.25 mL of beads was incubated with 2 mL of whole plasma for 30 min before observation.

Table III

Example #	Ligand	Attachment Reagent	Leukocytes per 1/2 bead				
			1	2	3	4	Total
1	Heparin (Kabi)	EEDQ	4	1	1	2	8
1	Heparin (Kabi)*	EEDQ	2	3	2	2	9
2	Heparin (Sigma)	EEDQ	2	1	0	0	3
3	Hyaluronic acid	EEDQ	0	0	0	0	0
4	Heparin (Kabi)	EDC	2	1	2	2	7
5	Heparin (Sigma)	EDC	1	1	1	2	5
6	Chondroitin sulfate A	EDC	0	1	1	0	2
11	Heparin (Kabi)	EEDQ	2	2	2	3	9
12	Heparin (Sigma)	EEDQ	3	3	2	2	10
13	Chondroitin sulfate A	EEDQ	1	1	2	0	4
14	Chondroitin sulfate B	EEDQ	2	1	0	0	3
15	Chondrosine	EEDQ	1	0	0	0	1
16	Hyaluronic acid	EEDQ	1	0	0	0	1
17	Heparin (Kabi)	EDC	1	0	0	0	1
18	Heparin (Sigma)	EDC	0	1	1	0	2
19	Chondroitin sulfate A	EDC	1	0	0	0	1
20	Chondroitin sulfate B	EDC	0	0	0	0	0
21	Chondrosine	EDC	0	1	0	0	1
22	Alginic acid	EDC	0	0	1	0	1
23	Hyaluronic acid	EDC	0	1	0	0	1

*In this experiment 0.02 mL of beads were used instead of 0.25 mL

The filters were examined under a phase contrast microscope (Zeiss) using an objective of 40x magnification and an eye piece of 10x magnification. At this magnification the leukocytes present in plasma (which are about 10 μm in diameter) appear as easily observable spheres, and can be distinguished

from platelets, which are more numerous. A small section of dry filter pad from Preparation A was removed from the inside of the pad, and placed on glass microscope slide, and several drops of plasma containing leukocytes was added, and allowed to mix for approximately 3 min. A cover glass was placed on the filter sample, and the filter examined for leukocyte binding to the fibers. The polyethylene fibers of the filter were approximately 8 μm and the cellulose acetate fibrils were approximately 1 μm in diameter. It was observed that several leukocytes bound to the cellulose acetate fibrils within the 5 to 20 min observation period under the microscope.

The heparin filters of Preparation A1 were also examined under the phase contrast microscope (Zeiss) using an objective of 40x magnification and an eye piece of 10x magnification. It was observed that several leukocytes bound to the cellulose acetate fibrils within the 5 to 20 min observation period under the microscope. The 44 hr hydrolyzed filter was found to bind the most number of leukocytes, whereas the 16 hr and 8 hr hydrolyzed filters appeared to bind a fewer number of leukocytes. The control unmodified fiber (Grade No. 825, Lot No. 3478, Roll No. 2-1-05) was also examined under the microscope for leukocyte binding using plasma.

The use of filter pads modified with heparin to remove leukocytes from whole blood was also examined. The results are shown in Table IV. When heparin was attached following oxidation of a plurality of hexose units in the heparin and condensation of the resulting aldehydes with aminated substrate, no measurable improvement in leukocyte capture was observed. On the other hand, when heparin was

attached by condensation of a glucuronic acid residue with aminated substrate, there was an increase in removal efficiency by one to two orders of magnitude. Reduction of leukocyte counts to the 100/mL range is clinically significant and is near the limit of detection.

Because of the nature of depth filtration, a series of filters can be "stacked" to increase overall efficiency of the unit. Thus, if one filter removes 90% of particles presented to it (of a certain type and size), two filters in series will remove 99% overall. By combining two untreated filters, to remove large debris and some leukocytes, and two heparin-containing filters, one can achieve very high efficiency in a small, cost effective device.

TABLE IV

Filter Pads	Attachment Chemistry	leukocytes/mL	
		Pre	Post
Control	None	4.1×10^7	6.4×10^3
Heparin	periodate	2.5×10^7	2.3×10^3
Control (4 pads)	None	1.1×10^7	7×10^3
Heparin (4 pads)	EEDQ	5×10^6	3×10^2
control (2) plus heparin (2)	EEDQ	8.3×10^6	1×10^2

E. Preparation of aminated chromatography beads with ATP covalently attached

Aminated Hyper D™ beads (available from Sepracor, Inc., Marlborough, MA) M grade (average size 85μm, 36μm eq/mL amino groups), 100g was placed in a 1L Nalgene plastic flask. Hyper D™ beads are porous silica beads having a gel composed of crosslinked aminated methacrylamide derivative in the

pores, as described in U.S. application serial number 07/956,404 filed October 5, 1992. One liter of oxidized ATP 5.5g (0.01M) prepared as described above in Example A2 was added to the flask, and mixed in a rotating mixer for 3 hours. At the end of 3 hours, the pH of the supernatant was adjusted to 7.4 using 10N sodium hydroxide, and mixed overnight. Solid sodium cyanoborohydride, 3.14 g (0.05M) was added to the flask, and mixed for an additional 4 hrs. The beads were transferred to a one liter sintered glass funnel (25-50 μm frit), washed extensively with water, and dried in air. Two other grades of aminated Hyper D beads were similarly derivatized with ATP.

F. Preparation of 2,3-Diphospho-D-glycerate (DPG) Filter Pads

The polypropylene/cellulose acetate filter pads were hydrolyzed with 1N NaOH for 44 hours and oxidized with sodium meta-periodate as described previously. A 1M solution of ethylenediamine (Aldrich Chemicals), pH 7.5, 1.5 liters, was made by adjusting the pH with concentrated HCl, and reacted with the oxidized pads (13) for 16 hours as described for adipic dihydrazide. Solid sodium cyanoborohydride (Sigma) was added to the ethylenediamine solution to bring the concentration to 0.1M in sodium cyanoborohydride, and reacted for an additional 4 hours. The filters were washed extensively with deionized water to remove excess cyanoborohydride and ethylenediamine, and dried overnight at 30° C.

2,3-Diphospho-D-glyceric acid, pentasodium salt, (DPG), 200 mg (Sigma) was dissolved in 50 ml of 0.1M MES buffer prepared by dissolving

1.92 g of 2-(N-morpholino)ethane sulfonic acid (Sigma Chemicals) in 100 mL deionized water and adjusting the pH to 4.5 with 1N sodium hydroxide solution. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC), 200 mg, (Sigma) was dissolved in 10 mL of 0.1M MES buffer and the pH adjusted to 4.5 with 1N sodium hydroxide. EDAC solution, 2.5 mL, was mixed with 50 mL of DPG solution for 1 minute, and gently poured onto two dry 90mm filter pads that had been modified with ethylenediamine. The pads were supported in a 1 L Nalgene plastic filter holder without the bottom membrane. After 15 minutes the DPG solution was removed from the filter pads by suction, and another 2.5 mL of EDAC solution was added gently to the filter pads on the filter. This procedure was repeated two more times until all the EDAC solution was added. The DPG/EDAC solutions were allowed to drain through the filter pads under gravity, and every 30 minutes the pads were dried by suction. The filtrate was gently poured on top of the pads and the process repeated for 7 more times. The filter pads were washed with 2 liters of 1M sodium chloride, and then extensively washed with deionized water to remove unreacted DPG and EDAC. The filters were dried in air at 30° C, and stored at 30° C .

Examples of the Second Apparatus Aspect and the First Embodiment of the Second Method Aspect

A series of devices have been developed which exemplify the second apparatus aspect of the invention. They incorporate multiple functions described above. These were evaluated for several

key performance criteria; extent of methylene blue removal, time for filtering a unit of plasma (the volume of a unit of single-donor plasma may vary from 200 to 300 mL), extent of leukodepletion and the effect of this filtration on the extent of depletion of various coagulation factors.

The filter device shown in Fig. 5 consists of a cylindrical housing **50** and cover **53** fitted with inlet **52** and outlet **54** tubing connectors. The housing holds layers of filter media 35 mm in diameter. The top layer **56** is a nonwoven fabric. Under this is a layer **58** of activated carbon/cellulose composite medium for methylene blue removal and up to four layers **60** of a nonwoven filter medium, which can be made from polypropylene, polyester, glass, and cellulose acetate components, for removal of leukocytes and lipids, and for polishing filtration. In the filter used in the examples, the carbon/cellulose composite was Carbac 2640FH™, available from Cellulo Company [Cranford, NJ] and the nonwoven filter for leukocytes was Type 825B from Lydall, Inc. [Hamptonville, NC].
The base of the cylindrical housing **50** has a spiral filtrate channel **62** to improve air removal and draining efficiency.

The filter device is connected at its inlet to a sealed, sterile-dockable tubing about 40 cm in length, and optionally a tubing clamp. The filter is connected from its outlet to a receiving blood bag with about 50 cm length of tubing. When the inlet tubing is sterile-docked to a supply bag of virally inactivated plasma, the distance measured from the midpoints of the supply and receiving bags is nominally 75 cm.

A unit of fresh-frozen plasma is thawed and brought to room temperature, and tare weighed. An aliquot of methylene blue solution is injected into the plasma corresponding to a final dye concentration of 0.1 μM (equivalent to 0.4 μg/mL). The plasma is mixed by manual agitation of the bag for about 30 seconds, and a sample is removed for analysis. This plasma supply bag is sterile-docked to the filter assembly via the clamped filter inlet tubing. The supply bag is hung on a stand, with the filter and supply bag suspended freely below. The tubing clamp is opened to start the flow of plasma. Total time for the entire unit of plasma to pass through the filter is monitored. The filtered plasma is sampled for analysis.

Examples of 1 to 10 utilized devices containing one layer of activated carbon filter and four layers of the nonwoven leukocyte filter; example 11 utilized two layers of leukocyte filter medium. Examples 1 to 4 illustrate methylene blue removal; examples 5 to 8 illustrate methylene blue removal and changes in coagulation factors before and after filtration; example 9 shows methylene blue removal and leukodepletion (equivalent to 99.94% removal); examples 10 and 11 show the effects of different filter configurations and plasma temperature variation on methylene blue removal and leukodepletion. For these tests pooled plasma was used immediately following thawing (temperature at start of filtration cycle was about 4°C).

Example	Plasma wt. (g)	Methylene blue concn. in filtered plasma (μg/mL) [a]	Filtration time (min)
1	300	<0.02	17.5
2	300	<0.02	14.9
3	205	<0.02	10.4
4	212	<0.02	10.6
5	264	<0.02	13.0
6	189	<0.02	11.3
7	199	<0.02	8.7
8	211	<0.02	13.5
9	169	<0.02	9.6
10	242	<0.02	39.6
11	287	<0.02	50.7

	Change in coagulation factor content (%)						Leukodepletion performance	
Example	Fibrino-gen	Factor					WBC count per mL before filtration	WBC count per mL after filtration [b]
		V	VII	VIII	IX	XI		
1								
2								
3								
4								
5	-3	2	5	-3	-15	-48		
6	0	-14	9	-6	-11	-54		
7	0	-3	8	-8	-11	-62		
8	-3	0	8	-6	-15	-53		
9							87800	50
10							7400	0
11							7400	0

[a]: 0.02 μg/mL is the limit of detection of methylene blue by HPLC

[b]: Nageotte method. (American Assoc. of Blood Banks Technical Manual; p 760; method 11.12)[end of 002b]

Claims

1. A filter comprising a laid textile web which includes a mechanically stable polymeric substrate, at least a portion of said polymeric substrate having covalently attached thereto a first ligand, said first ligand having affinity for viral inactivating agents.

2. A filter according to claim 1 wherein said polymeric substrate has additionally covalently attached thereto a second ligand, said second ligand having affinity for a leukocyte cell surface.

3. A filter for removing leukocytes from whole blood or a blood fraction, comprising a laid textile web which includes a mechanically stable polymeric substrate, at least a portion of said polymeric substrate having covalently attached thereto a ligand having affinity for the leukocyte cell surface.

4. A filter according to any of claims 1-3 wherein said ligand is attached directly to said polymeric substrate.

5. A filter according to any of claims 1-3 wherein said ligand is attached to said polymeric substrate through at least one intervening linker.

6. A filter according to any of claims 1-3 wherein an intervening linker is the residue of an alkylene diamine.

7. A filter according to claim 6 wherein said linker is attached to said ligand by an amide bond to a carboxyl of said ligand.

8. A filter according to claim 1 or 2 wherein said first ligand is a hydrazine-terminated moiety.

9. A filter according to claim 1 or 2 wherein said first ligand is a mimic of a viral DNA or RNA.

10. A filter according to claim 2 or 3 wherein a ligand is a carbohydrate.

11. A filter according to claim 10 wherein said ligand is a sulfoglycan that includes residues of glucuronic acid.

12. A filter according to claim 11 wherein said ligand is heparin.

13. A filter according to any of claims 1 to 3, comprising a shape-sustaining laid textile web having a thickness of 1 to 8 mm and a bulk density of 0.05 to 0.4 g/cm^3, said web comprising:

(a) a plurality of interlocked textile fibers with average deniers between 0.05 and 0.75 and average lengths between 3 mm and 15 mm, said textile fibers being substantially uniformly distributed in said web so as to form a matrix of the textile fibers with spaces between adjacent interstices of interlocked fibers; and

(b) a plurality of fibrillated particles of surface-modified polymeric material having a surface area of 5 to 60 square meters per gram substantially disposed within said spaces of the

matrix, said fibrillated particles having a plurality of fine fibrils which are interlocked with adjacent textile fibers of said spaces such that the fibrillated particles are not substantially displaceable from said web during filtration of said blood;

wherein the weight ratio of the fibrillated particles to the textile fibers is between 1:99 and 40:60; and wherein said surface-modified polymeric material is a hemolytically inert polymer covalently linked to one or more ligands, said ligands having an affinity for leukocytes, viral inactivating agents, or both; and wherein said textile fibers are hemolytically inert.

14. A filter according to claim 13 wherein said surface-modified polymeric material is cellulose acetate.

15. A filter according to claim 13 wherein said textile fibers are one or more of polyolefin, polyamide, polysulfone, polyester, polyvinyl alcohol, and poly(ethylene-vinyl alcohol) copolymer fibers.

16. A hollow-fiber or flat-sheet membrane for removing leukocytes, viral inactivating agents, or both, from a blood fraction or whole blood comprising:

(a) polyethersulfone (PES) as the primary hydrophobic polymer component, said PES having functionalizable phenolic chain ends;

(b) a first linker moiety attached to a plurality of said phenolic chain ends, said first linker derived from an oxirane selected from the group consisting of ethylene glycol diglycidyl ether, 1,4-butanediol diglycidyl ether and epichlorohydrin; and

(c) a surface-modified polymeric material attached to a plurality of said first linker moieties, said surface-modified polymeric material consisting of a hemolytically inert polymer covalently attached to a plurality of ligands for viral inactivating agents, for leukocytes, or for both.

17. A hollow-fiber or flat-sheet membrane according to claim 16 wherein said surface-modified polymeric material is a hydroxyalkylcellulose or polyethyleneimine covalently attached to said plurality of ligands through a second linker moiety.

18. A hollow-fiber or flat-sheet membrane according to claim 16 wherein one of said ligands is heparin.

19. A hollow-fiber or flat-sheet membrane according to claim 16 wherein said ligands are heparin and polythymidine.

20. A method for removing one or more viral inactivating agents from whole blood or a blood fraction comprising passing said blood or blood fraction through a filter according to claim 1 or 2.

21. A method for removing leukocytes from whole blood or a blood fraction comprising passing said blood through a filter according to claim 2 or 3.

22. A method for simultaneously removing leukocytes and one or more viral inactivating agents from whole blood or a blood fraction comprising passing said blood or blood fraction through a filter according to claim 2.

23. A method for removing viral inactivating agents from whole blood or a blood fraction comprising providing flowing contact between said blood and a hollow-fiber membrane according to any of claims 16-19.

24. A method for removing leukocytes from whole blood or a blood fraction comprising providing flowing contact between said blood and a hollow-fiber membrane according to any of claims 16-19.

25. A method for removing both leukocytes and viral inactivating agents from whole blood or a blood fraction comprising providing flowing contact between said blood and a hollow-fiber membrane according to any of claims 16-19.

26. A method for producing a viral inactivating agent filter comprising:

(a) providing a shape-sustaining web comprising:

(1) a plurality of fibers that are resistant to alkaline degradation; and

(2) a plurality of cellulose acetate fibers;

(b) treating said web with aqueous base to hydrolyze a portion of cellulose acetate esters to the corresponding free hydroxyls;

(c) activating said free hydroxyls to produce amine-reactive residues;

(d) reacting said amine-reactive residues with a diamine or a dihydrazide; and

(e) reacting the resulting amine or hydrazide with an activated derivative of a ligand for a viral inactivating agent.

27. A method for producing a filter according to claim 26 wherein in step (e) the resulting amine or hydrazide is additionally reacted with an activated derivative of a ligand for leukocytes.

28. A method for producing a leukocyte filter comprising:

(a) providing a shape-sustaining web comprising:

(1) a plurality of fibers that are resistant to alkaline degradation; and

(2) a plurality of cellulose acetate fibers;

(b) treating said web with aqueous base to hydrolyze a portion of cellulose acetate esters to the corresponding free hydroxyls;

(c) activating said free hydroxyls to produce amine-reactive residues;

(d) reacting said amine-reactive residues with a diamine; and

(e) reacting the resulting amine with an activated derivative of a carbohydrate carboxylic acid.

29. A method for producing a filter according to any of claims 26-28 comprising:

(a) providing a shape-sustaining laid textile web having a thickness of 1 to 8 mm and a bulk density of 0.05 to 0.4 g/cm^3, said web comprising:

(1) a plurality of interlocked polyolefin fibers with average deniers between 0.05 and 0.75 and average lengths between 3 mm and 15 mm, said polyolefin fibers being substantially uniformly distributed in said web so as to form a

matrix of the polyolefin fibers with spaces between adjacent interstices of interlocked fibers; and

(2) a plurality of fibrillated cellulose acetate particles having a surface area of 5 to 60 square meters per gram substantially disposed within said spaces of the matrix, said fibrillated particles having a plurality of fine fibrils which are interlocked with adjacent polyolefin fibers of said spaces such that the fibrillated particles are not substantially displaceable from said web during filtration of blood, wherein the weight ratio of the fibrillated particles to the polyolefin fibers is between 1:99 and 40:60; and

(b) treating said web with aqueous base to hydrolyze a portion of cellulose acetate esters to the corresponding free hydroxyls;

(c) activating said free hydroxyls to produce amine-reactive residues;

(d) reacting said amine-reactive residues with a diamine or a dihydrazide; and

(e) reacting the resulting amine or hydrazide with an activated derivative of a ligand chosen from the group consisting of polythymidine, heparin and mixtures of the two.

30. A method according to claim 29 wherein said web is treated with 1 N aqueous sodium or potassium hydroxide at 20 to 40°C for 8 to 312 hours.

31. A method according to claim 29 wherein said free hydroxyls are activated by reaction with an activating agent selected from the group consisting

of: cyanogen bromide, carbonyl diimidazole, divinyl sulfone, azlactones, sulfonyl chlorides, diepoxides, dihalides, haloepoxides, 2,4,6-trichloro-S-triazine, 2-fluoro-1-methylpyridium salts, disulfonyl chlorides, periodate, diacid chlorides, diisocyanates, and haloacetic acid followed by N-hydroxysuccinimide plus a carbodiimide.

32. A method according to claim 29 wherein said amine-reactive residues are reacted with adipic dihydrazide to produce a plurality of N-monosubstituted hydrazides.

33. A method according to claim 29 wherein said free hydroxyls are activated by reaction with periodate.

34. A method for simultaneously removing leukocytes and one or more viral inactivating agents from whole blood or a blood fraction comprising passing said blood or blood fraction through a filter adapted for removing leukocytes and antiviral agents, said filter comprising a mechanically stable polymeric material capable of retaining leukocytes and activated carbon capable of removing said viral inactivating agent.

35. A method according to claim 34 wherein said blood fraction is plasma.

36. A method according to claim 34 wherein said viral inactivating agent is selected from the group consisting of phenothiazine dyes and photo-decomposition products of phenothiazine dyes.

37. A method according to claim 36 wherein said viral inactivating agent is selected from the group consisting of methylene blue, toluidine blue, and photodecomposition products of methylene blue and toluidine blue.

38. A filter device for removing leukocytes and one or more viral inactivating agents from whole blood or a blood fraction, comprising (1) a housing, enclosing (2) an activated carbon-containing filter element and (3) at least one filter element adapted for retaining leukocytes.

39. A filter device for removing leukocytes and one or more viral inactivating agents from whole blood or a blood fraction, comprising (1) a housing, enclosing (2) an activated carbon-containing filter element and (3) at least one filter element according to any of claims 1-3.

40. A filter device according to claim 38 wherein said activated carbon-containing filter element comprises an activated carbon-impregnated cellulose composite medium.

41. A method for simultaneously removing leukocytes and one or more viral inactivating agents from whole blood or a blood fraction comprising passing said blood or blood fraction through a filter according to claim 38.

42. A method for simultaneously removing leukocytes and one or more viral inactivating agents from whole blood or a blood fraction comprising passing said blood or blood fraction through a filter according to any of claim 39.

FIG. 1

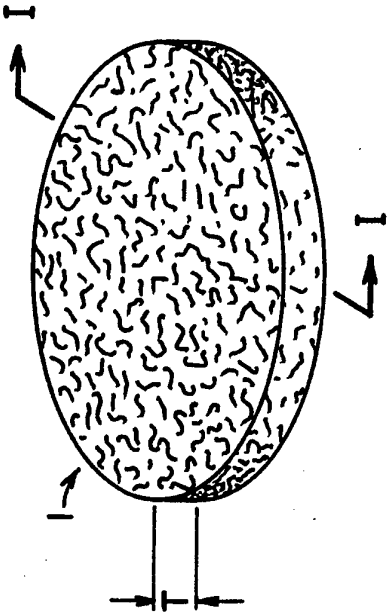


FIG. 2

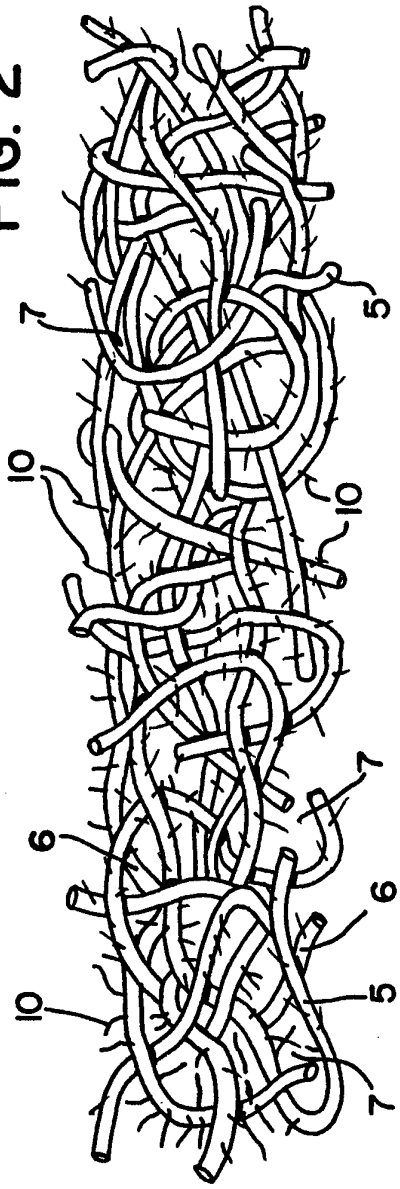


FIG. 3

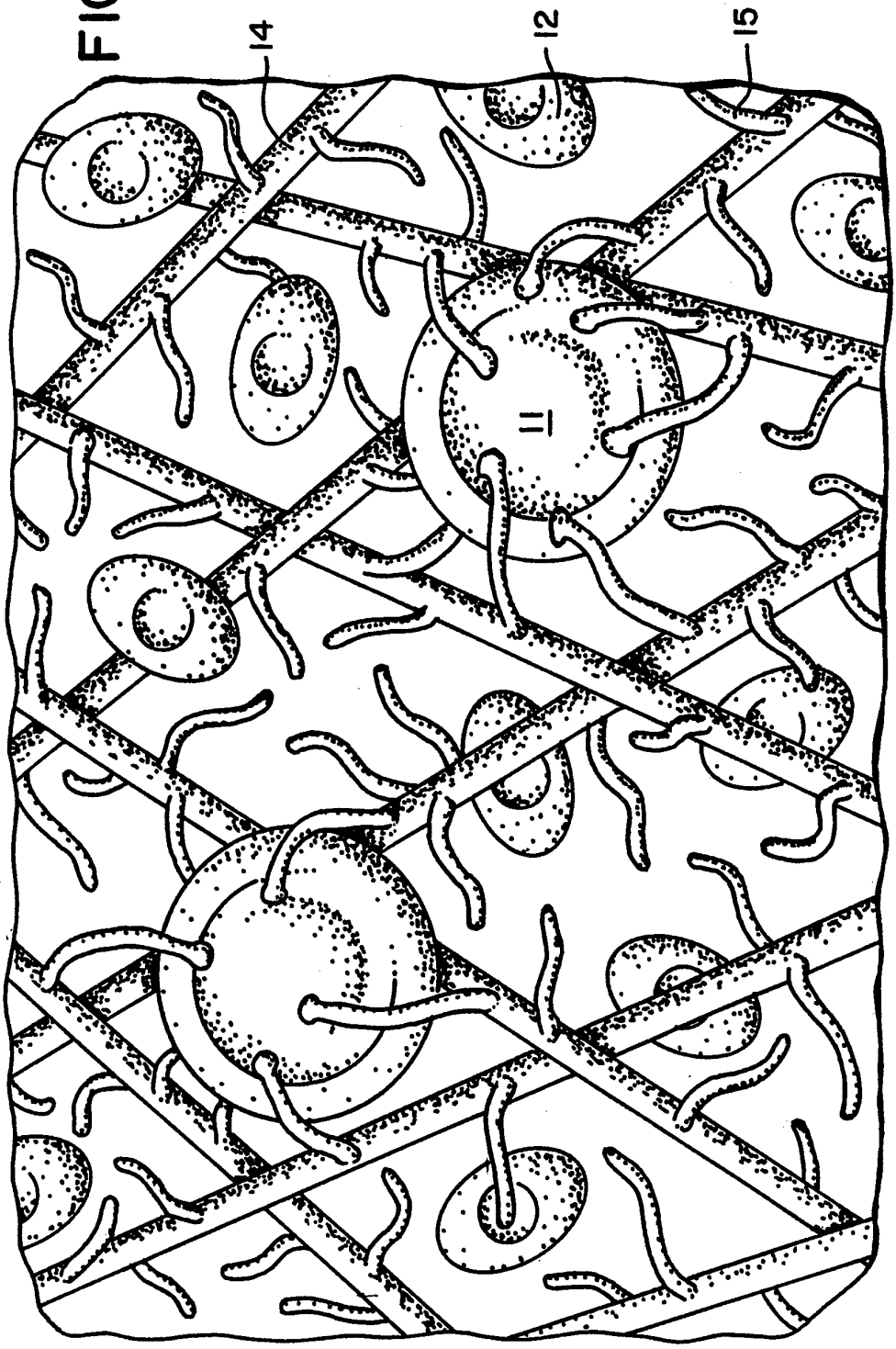
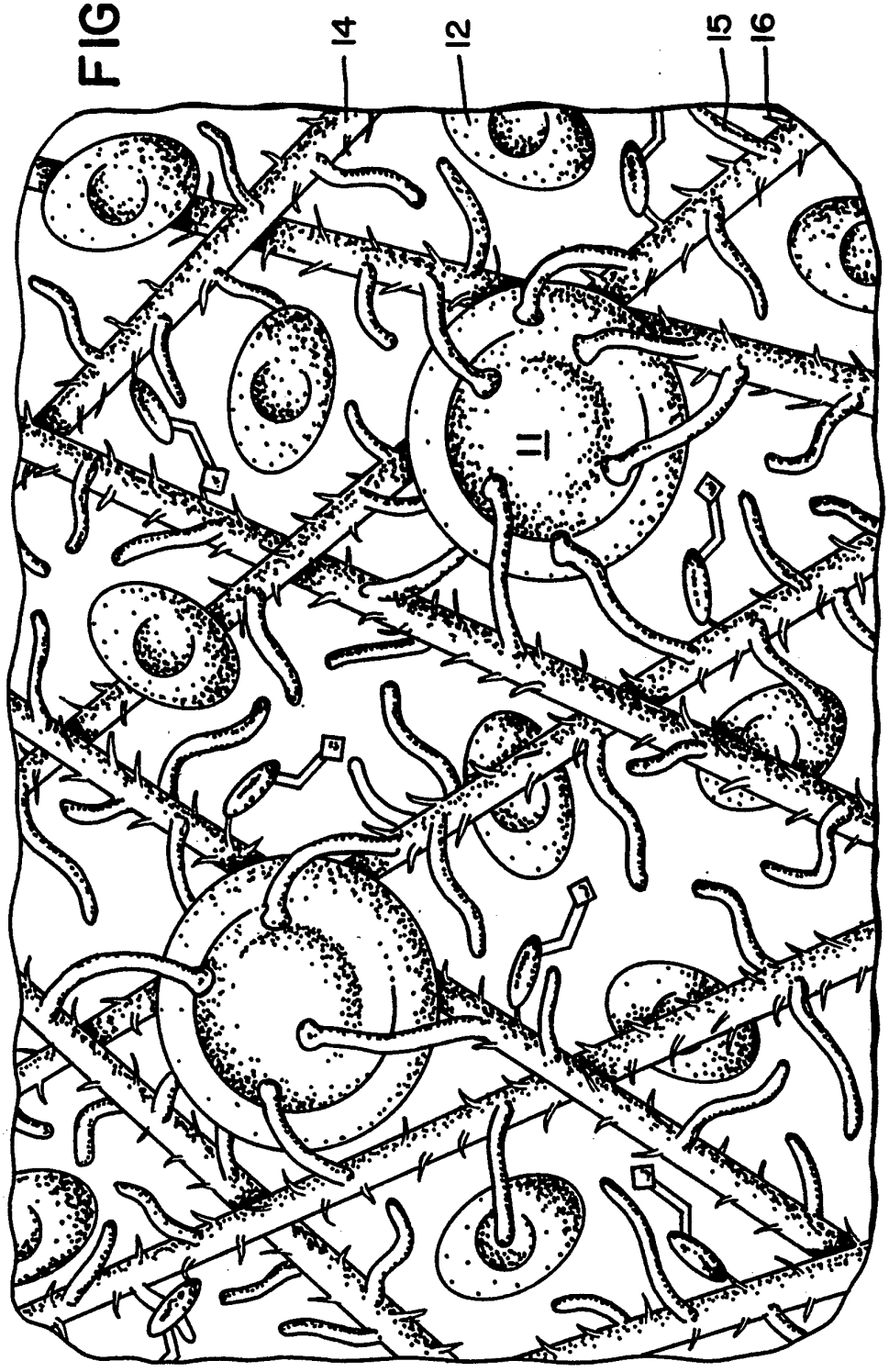


FIG. 4



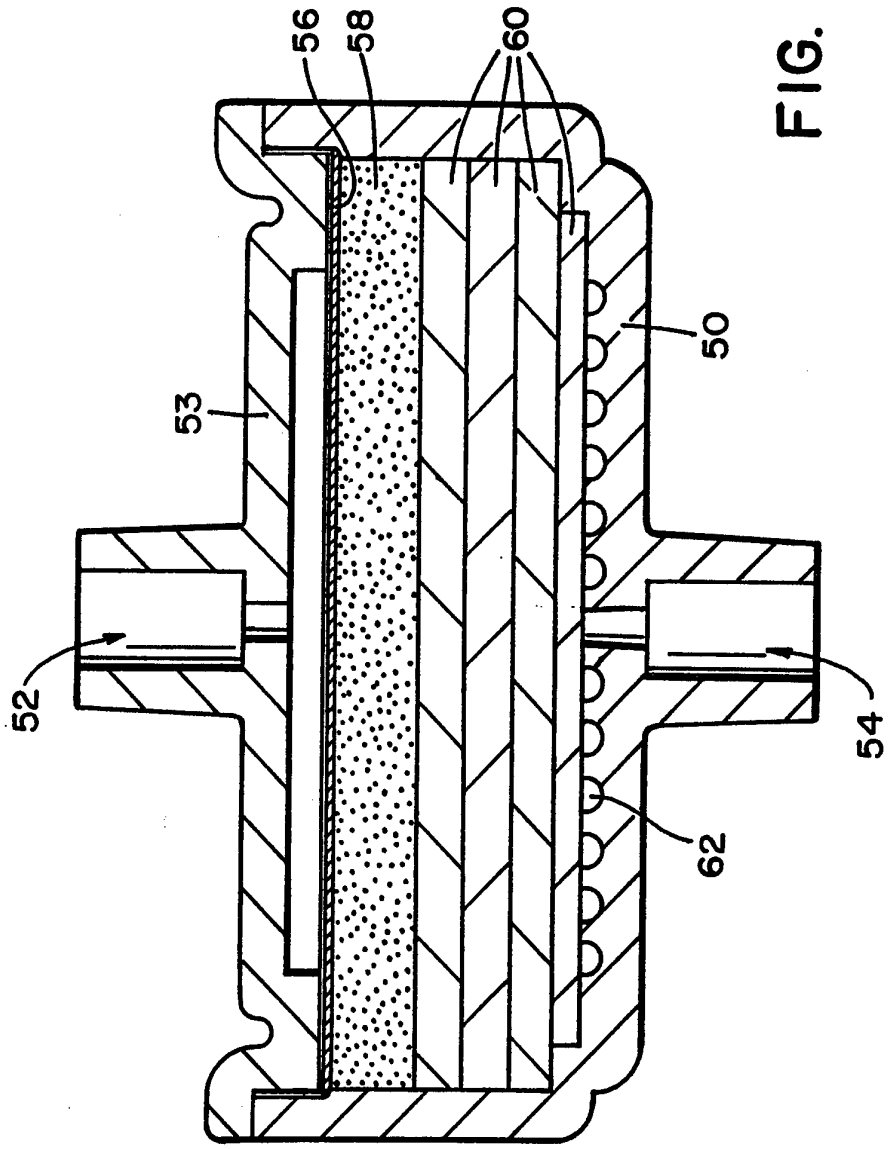


FIG. 5

INTERNATIONAL SEARCH REPORT

national Application No
PCT/US 95/00355

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 B01D39/16 B01D67/00

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)
IPC 6 B01D G01N A61M

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 practical, 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.
A	WO,A,90 05018 (SEPRACOR, INC.) 17 May 1990 see page 1, paragraph 2 see page 37, line 25 - page 44, line 23 --- -/--	2-5, 9-12, 15-19, 21-25, 29,31,35

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in 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 document but 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
30 May 1995	16.06.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Stevnsborg, N

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

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 173 500 (PALL CORPORATION) 5 March 1986 see abstract see page 1, paragraph 1 see page 3, paragraph 2 see page 7, paragraph 2 - page 9, paragraph 1 see page 15, paragraph 3 - page 16, paragraph 1 see page 20, paragraph 2 - page 23, paragraph 1 see table 1	2-4, 8-13,15, 16,18, 19,21, 24,25, 28,29, 31,34,35
A	WO,A,91 03933 (BLUTSPENDEDIENST DER LANDESVERBÄNDE DES DEUTSCHEN ROTEN KREUZES) 20 April 1994 see abstract see claims; example 13	1,20,22, 34-37
A	US,A,5 190 657 (LYDALL, INC.) 2 March 1993 cited in the application see the whole document	13-15, 26,28,29
A	WO,A,93 16176 (BIO-METRIC SYSTEMS, INC.) 19 August 1993 see the whole document	1-42
A	EP,A,0 341 413 (ASAHI MEDICAL CO., LTD.) 15 November 1989 see the whole document	1-42

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No
PCT/US 95/00355

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9005018	17-05-90	AT-T-	106272	15-06-94
		AU-A-	4524789	28-05-90
		CA-A-	2001720	30-04-90
		DE-D-	68915788	07-07-94
		DE-T-	68915788	20-10-94
		DE-T-	483143	17-03-94
		EP-A-	0483143	06-05-92
		US-A-	5310688	10-05-94
EP-A-0173500	05-03-86	US-A-	4693985	15-09-87
		CA-A-	1249781	07-02-89
		DE-A-	3585542	09-04-92
		EP-A,B	0280840	07-09-88
		GB-A,B	2163434	26-02-86
		GB-A,B	2199327	06-07-88
		JP-B-	6089169	09-11-94
		JP-A-	61124868	12-06-86
WO-A-9103933	04-04-91	DE-A-	3930510	21-03-91
		AU-B-	635068	11-03-93
		AU-A-	6339190	18-04-91
		EP-A-	0491757	01-07-92
		EP-A-	0593098	20-04-94
		JP-T-	5500366	28-01-93
US-A-5190657	02-03-93	CA-A-	2102299	23-01-93
		EP-A-	0595899	11-05-94
		JP-T-	7500090	05-01-95
		US-A-	5290449	01-03-94
		WO-A-	9301880	04-02-93
WO-A-9316176	19-08-93	AU-B-	3664693	03-09-93
		CA-A-	2107683	14-08-93
		EP-A-	0585436	09-03-94
		JP-T-	6506959	04-08-94
EP-A-0341413	15-11-89	AU-A-	3237489	05-10-89
		DE-D-	68910175	02-12-93
		DE-T-	68910175	17-02-94
		JP-A-	2029260	31-01-90

INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No
PCT/US 95/00355

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0341413		US-A- 5286449	15-02-94