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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 October 2015 (29.10.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/164198 A1

(51) International Patent Classification:
A61M 1/36 (2006.01)

(21) International Application Number:
PCT/US2015/026340

(22) International Filing Date:
17 April 2015 (17.04.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/984,013 24 April 2014 (24.04.2014) US

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2015/164198 A1

(54) Title: METHOD FOR REMOVING BACTERIA FROM BLOOD USING HIGH FLOW RATE

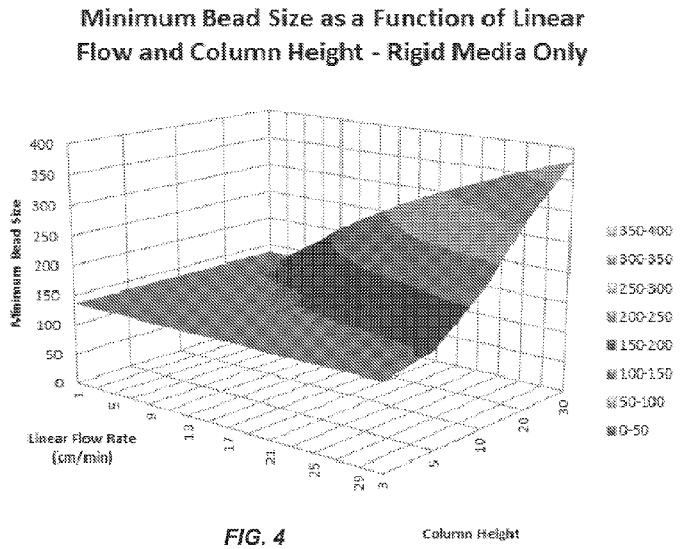


FIG. 4

(57) Abstract: The present invention provides methods for removing a significant amount of bacteria (e.g., gram-negative bacteria and gram-positive bacteria, including bacteria with no or low affinity for heparan sulfate) from whole blood, serum or plasma using an adsorption media. The method can be used in extracorporeal treatments involving high volumetric flow rates and high linear flow rates.

METHOD FOR REMOVING BACTERIA FROM BLOOD USING HIGH FLOW RATE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to US Provisional Patent Application No. 61/984,013, filed April 24, 2014, the teachings of which are hereby incorporated by reference in their entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] Bloodstream infection, or bacteremia, is a major challenge in the Intensive Care Unit (ICU). Bacteremia can quickly lead to septic shock, meningitis, endocarditis, osteomyelitis and other metastatic complications. *Staphylococcus aureus*, *P. aeruginosa* and *Enterobacteriacea* are the most common bacteria responsible for bacteremia and nosocomial infections. Severity of outcome for bacteremic patients is correlated to both the bacterial load and duration of bacteremia. For example, a quantitative RT-PCR study of *E. coli* and *S. aureus* bacteremia patients showed that when the number of rDNA increased to over 1,238 copies/ml, mortality increased from 14.3% to 42.9% and septic shock increased from 31.4% to 85.7%. It was also found that a high blood concentration of *N. meningitides* is correlated with prolonged hospitalization, limb or tissue loss, the need for dialysis, and patient mortality. Another study showed that the severity of *Pneumococcal* pneumonia correlated with bacterial load in the blood: the mortality for patients with over 1000 *S. pneumoniae* DNA copies/ml of blood was 25.9% vs. 6.1% for patients exhibiting less than 1000 copies/ml. In yet another study, a follow-up positive blood culture between 48 and 96 hours after initial diagnosis was shown to be the strongest predictor of complicated *S. aureus* bacteremia. Compounding the difficulty of effective bacteremia treatment is the often delayed administration of appropriate antibiotic therapy. For each hour of delay in treatment the mortality risk increases over 7%.

[0003] The conventional strategy for combating bacterial infections is to administer active drugs that specifically kill bacteria while minimizing damage to host tissue. This is a major

challenge as some of the more effective antibiotics available today are quite toxic. For example, vancomycin is nephrotoxic, and may soon be contraindicated for patients undergoing extracorporeal oxygenation. Even if new antibiotics are successfully developed to address current drug resistance, new superbugs will continue to emerge. Clearly, new strategies for combating infection are needed, in addition to drug discovery.

[0004] Drug-resistant pathogens are a growing threat to the healthcare system. The CDC has recently warned of the emergence of carbapenem-resistant *Enterobacteriacea* (CRE; "super bugs"). The mortality rate for CRE bacteremia can be as high as 50%. Resistance of CREs to even the strongest available antibiotics leaves clinicians with few treatment options. The incidence of hospital-acquired CRE infections has increased 400% over the last 10 years. Currently, CRE bacteremias are mostly nosocomial infections, but there is concern that the incidence of community acquired CRE could increase. Today, the only strategy is to reduce CRE infections is through education and prevention.

[0005] There is a need for a safe, broad-spectrum technology that can quickly reduce bacterial load, and shorten the duration of bacteremia. The present invention satisfies this and other needs by providing a high-surface-area extracorporeal affinity adsorption media that can quickly and safely remove pathogens from whole blood or whole serum.

BRIEF SUMMARY OF THE INVENTION

[0006] The present invention provides methods that can quickly reduce bacterial load, and shorten the duration of bacteremia even without first identifying the type of bacteria present in the blood.

[0007] In some aspects, provided herein is an *ex vivo* method for removing bacteria from a sample taken from a subject who is suspected of being infected with bacteria. The method comprising, consisting essentially of or consisting of: contacting a sample taken from the subject with an adsorption media to allow the formation of an adhering complex, wherein the adhering complex comprises bacteria and the adsorption media; and separating the sample from the adhering complex to produce the sample with a reduced amount of bacteria. Typically, the adsorption media is contained within a column, a container or cartridge.

[0008] In some embodiments, the sample is selected from the group consisting of whole blood, serum and plasma. In other embodiments, the sample is whole blood.

[0009] In some embodiments, the adsorption media is a solid substrate of high surface area having a hydrophilic surface that is free of a polysaccharide adsorbent. In some instances, the solid substrate comprises a plurality of rigid polymer bead. In some embodiments, the rigid polymer bead is a member selected from the group consisting of polyurethane, polymethylmethacrylate, polyethylene or co-polymers of ethylene and other monomers, polyethylene imine, polypropylene, and polyisobutylene. In other embodiments, the solid substrate comprises one or a plurality of hollow fibers or yarn.

[0010] In some embodiments, the hydrophilic surface is a cationic surface. In other embodiments, the hydrophilic surface is a neutrally charged surface.

[0011] In some embodiments, the bacteria in the sample are reduced by about 20% to about 99.9%. In other embodiments, the bacteria in the sample are reduced by about 20% to about 40%.

[0012] In some embodiments, the bacterium is a gram-negative bacterium. In other embodiments, the bacterium is a gram-positive bacterium. In other embodiments, the bacteria is selected from the group consisting of *Escherichia coli*, *Klebsiella pneumoniae*, carbapenem-resistant *Escherichia coli*, carbapenem-resistant *Klebsiella pneumoniae*, and extended spectrum beta-lactamase *Klebsiella pneumoniae*, *Enterococcus* faecium, *Acinetobacter baumannii*, and methicillin-resistant *Staphylococcus aureus* (MRSA). In yet other embodiments, the bacterium is selected from the group consisting of *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Escherichia coli*.

[0013] In some embodiments, the cationic surface of the adsorption media forms an adhering complex with bacteria selected from the group consisting of *Escherichia coli*, *Klebsiella pneumoniae*, carbapenem-resistant *Escherichia coli*, carbapenem-resistant *Klebsiella pneumoniae*, and extended spectrum beta-lactamase *Klebsiella pneumoniae*, *Enterococcus* faecium, *Acinetobacter baumannii*, and methicillin-resistant *Staphylococcus aureus* (MRSA). In other embodiments, the neutrally charged surface forms an adhering complex with bacteria

selected from the group consisting of *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Escherichia coli*.

[0014] In some aspects, provided herein is an *ex vivo* method for removing bacteria from a sample taken from a subject who is suspected of being infected with bacteria, wherein the bacteria are known to have a low affinity or no affinity for heparan sulfate. The method comprising, consisting essentially of or consisting of: contacting a sample taken from a subject with an adsorption media to allow the formation of an adhering complex, wherein the adsorption media is a solid substrate of high surface area having at least one polysaccharide adsorbent on the surface thereof and separating the sample from the adhering complex to produce the sample with a reduced amount of bacteria. The adhering complex comprises bacteria and the adsorption media. Typically, the adsorption media is contained within a column, a container or cartridge. In certain aspects, the sample exits the column, the container or the cartridge, and the adhering complex remains behind.

[0015] In some embodiments, the sample is selected from the group consisting of whole blood, serum and plasma. In other embodiments, the sample is whole blood.

[0016] In some embodiments, the solid substrate comprises a plurality of rigid polymer bead. In some instances, the rigid polymer bead is a member selected from the group consisting of polyurethane, polymethylmethacrylate, polyethylene or co-polymers of ethylene and other monomers, polyethylene imine, polypropylene, and polyisobutylene. In other embodiments, the solid substrate comprises one or a plurality of hollow fibers.

[0017] In some embodiments, the at least polysaccharide absorbent is a member selected from the group consisting of heparin, heparan sulfate, hyaluronic acid, sialic acid, carbohydrates with mannose sequences, and chitosan. In other embodiments, the at least polysaccharide absorbent is heparin or heparan sulfate. In some instances, the at least polysaccharide absorbent is heparin.

[0018] In some embodiments, the beads are coated with about 0.27 mg to about 10 mg heparin per gram of bead. In other embodiments, the bead is coated with 2 ±0.5 mg heparin per gram of bead.

[0019] In some embodiments, the bacteria in the sample are reduced by about 20% to about 99.9%. In other embodiments, the bacteria in the sample are reduced by about 20% to about 40%.

[0020] In some embodiments, the bacteria are gram-negative bacteria. In other embodiments, the bacteria are gram-positive bacteria. In yet other embodiments, the bacteria is selected from the group consisting of *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterococcus faecium*, carbapenem-resistant *Escherichia coli*, carbapenem-resistant *Klebsiella pneumoniae*, and extended spectrum beta-lactamase *Klebsiella pneumoniae*.

[0021] In some aspects, provided herein is an *ex vivo* method for removing bacteria from a sample taken from a subject undergoing dialysis or extracorporeal oxygenation. The method comprising, consisting essentially of, or consisting of: contacting a sample taken from a subject with an adsorption cartridge comprising adsorption media, wherein the adsorption cartridge is in series with a dialysis cartridge or oxygenator to allow the formation of an adhering complex and separating the sample from the adhering complex to produce the sample with a reduced amount of bacteria. The adhering complex comprises bacteria and adsorption media. Typically, the adsorption media is contained within a column, a container or cartridge. In certain aspects, the sample exits the column, the container or the cartridge, and the adhering complex remains behind.

[0022] In some embodiments, the sample has a total blood volume of less than 200 ml.

[0023] In some embodiments, the adsorption cartridge has a column height between 1 cm-50 cm. In some embodiments, the adsorption cartridge has a column diameter between 1 cm-50 cm.

[0024] In some embodiments, the adsorption cartridge is proximal to the subject compared to the dialysis cartridge. In other embodiments, the adsorption cartridge is distal to the subject compared to the dialysis cartridge.

[0025] These and other aspects, objects and advantages will become more apparent when read with the figures and the detailed description which follow.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] FIGS. 1A-B show a comparison of the adsorption media and human blood. FIG. 1A shows the adsorption media and FIG. 1B shows an image of a human blood smear.

[0027] FIG. 2 shows a size comparison of bacteria, *e.g.*, *Staphylococcus aureus* and *Chlamydia*, and viruses, *e.g.*, pox virus, herpes virus, influenza virus, and picornavirus (polio).

[0028] FIG. 3 illustrates a cross-section of the adsorption media containing beads with a diameter (d) and a cell with a diameter (a).

[0029] FIG. 4 illustrates the minimum bead size as a function of linear flow and adsorption cartridge column height for a rigid media subject to forced convection.

DETAILED DESCRIPTION OF THE INVENTION

[0030] The present invention is based, in part, on the discovery of an adsorption media that is effective for removing a significant amount of bacteria (*e.g.*, gram-negative bacteria and gram-positive bacteria, including bacteria with no known affinity or low affinity for heparan sulfate) from blood (*e.g.*, whole blood and blood serum). In addition, the adsorption media can be used in extracorporeal treatments involving high volumetric flow rates and high linear flow rates. Typically, the adsorption media is contained within a column, a container or cartridge. In certain aspects, the sample exits the column, the container or the cartridge, and an adhering complex remains behind.

[0031] A first aspect of the present invention provides a method for the removal of bacteria from blood, such as mammalian blood, by contacting the blood with a solid substrate. The inventors have found that the surface architecture of the solid substrate is effective for removing pathogens such bacterial pathogens or viruses.

[0032] The substrate of the present invention possesses sufficiently large interstitial dimensions to permit a high flow rate of blood over the substrate without a large pressure drop. For instance, as blood is taken from a mammalian patient, it is passed over the substrate at a flow rate whereby the delivery of adsorbates to the surface of the adsorbent bed is characterized primarily by forced convection. Substrates suited for convection transport, generally rely on

macroscopic "channels" or visible interstices between solid, essential nonporous material, such as particles, beads, fibers, yarn, reticulated foams, or optionally spiral-wound dense membranes.

[0033] This is in contrast to highly porous adsorbent media (*e.g.*, porous silica, Sephadex®, crosslinked polystyrene and other size exclusion media), and many other microporous media that use the much slower process of molecular diffusion. Adsorption substrates that depend on diffusion transport are generally composed of porous materials with microscopic pores and an extremely high internal surface area.

I. Definitions

[0034] The term "extracorporeal therapy" includes a medical procedure that is conducted outside the body i.e., *ex vivo*. In some instances, extracorporeal therapies include methods in which a bodily fluid such as blood is taken from the individual and desired products such as, but not limited to, oxygen, blood-anticoagulants, anesthetics, and the like are added to the body fluid before it is returned to the individual. In other instances, an extracorporeal therapy includes removing undesired products like naturally occurring toxins, poisons or viruses from the body or the body fluids. Non-limiting examples of extracorporeal therapies include apheresis, autotransfusion, hemodialysis, hemofiltration, plasmapheresis, extracorporeal circulation (ECC), extracorporeal life support (ECLS) extracorporeal membrane oxygenation (ECMO), and cardiopulmonary bypass.

[0035] The term "high flow rate" or "high flow condition" includes a flow rate or velocity of blood that is above the diffusion limit.

[0036] The term "adsorption media" includes a material to which a cell, organism, virus, pathogen, polypeptide, polynucleotide, chemical molecule, biological molecule can adhere to the surface thereof and be removed from a sample such as blood.

[0037] The term "adhering complex" includes a complex of at least two molecules wherein the first molecule is attached (*e.g.*, linked, coupled or bound) to a surface such as a substrate and the second molecule is attached to the first molecule. For example, a pathogen or virus can adhere to heparin to form an adhering complex. Typically, in the methods of the present invention, the adhering complex remains behind and the sample is cleansed of the patthogen or virus.

[0038] The term "high surface area" includes the property of having a large specific surface area to volume ratio.

[0039] The term "adsorbent" includes a solid substrate with a chemical compound, a biological molecule, or a material that is attached (*e.g.*, linked, coupled or bound) thereto. In certain instances, the adsorbent is the solid substrate itself. In one embodiment, an adsorbent is a polymer resin with a polysaccharide such as heparin bound thereto. The substrate can be a polymer bead, fiber or yarn.

[0040] The term "rigid polymer bead" refers to a bead, granule, pellet, sphere, particle, microcapsule, sphere, microsphere, nanosphere, microbead, nanobead, microparticle, nanoparticle, and the like that is made from a polymer resin. A polymer bead is useful as a substrate.

[0041] The term "fiber" or "yarn" is useful as a soild substrate. The fiber or yarn can be made of a synthetic polymer or a natural polymer or a mixture thereof. In certain instances, an originally porous hollow fiber or yarn is rendered dense or non-porous before, during or after binding heparin or other adsorbents to the outer and/or inner surfaces thereof.

[0042] The term "carbohydrate" refers to a molecule containing carbon, hydrogen and oxygen atoms, and usually with the empirical formula $C_x(H_2O)_y$, where x and y are different numbers. Examples of carbohydrates includes monosaccharides, disaccharides, oligosaccharides, and polysaccharides.

[0043] The term "polysaccharide" refers to a molecule of monosaccharide units joined together by glycosidic bonds, and having an empirical formula of $C_x(H_2O)_y$, where x is between 200 to about 3000.

[0044] The term "hydrophilic surface" includes a surface with a water contact angle less than 90^o when the surface is flat.

[0045] The term "low affinity to heparan sulfate" in the context of a bacteria, refers to the low binding affinity of the bacteria for heparan sulfate. In some embodiments, the binding affinity is determined using standard assays, such as an enzyme-linked immunosorbent assay (ELISA) for heparan sulfate. In other embodiments, the binding affinity is determined based on a predictive

analysis, such as an analysis of putative heparan sulfate binding proteins expressed by the pathogen, e.g., bacteria. The term "no affinity for heparan sulfate" refers to a bacteria having no binding affinity for or a lower than detectable affinity for heparan sulfate, or no known binding to heparan sulfate. In some instances, having no affinity for heparan sulfate includes having no predicted binding affinity for heparan sulfate.

II. Detailed Description of Embodiments

A. Binding of Bacterial Pathogens by Convection Transport

[0046] The binding of bacterial pathogens to the essentially nonporous adsorption substrate of the present invention during convection transport is particularly effective under the relatively high-flow conditions typically employed in the safe operation of extracorporeal blood circuits, *e.g.* when measured by linear flow velocity, ≥8 cm/min, preferably about ≥30 cm/min, and more preferably about 30-1,000 cm/min.

[0047] In some embodiments, the adsorption media removes pathogens from whole blood in extracorporeal circuits with a linear flow rate of about 8 cm/min to about 1,000 cm/min, *e.g.*, about 8 cm/min to about 30 cm/min, about 25 cm/min to about 100 cm/min, about 50 cm/min to about 200 cm/min, about 100 cm/min to about 1000 cm/min, about 200 cm/min to about 1000 cm/min, about 400 cm/min to about 1000 cm/min, about 500 cm/min to about 1000 cm/min, about 600 cm/min to about 1000 cm/min, about 100 cm/min to about 500 cm/min or about 300 cm/min to about 800 cm/min. In certain instances, the flow rate is about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 100 cm/min or about 25-40 cm/min.

[0048] In other embodiments, the adsorption media removes pathogens from whole blood in extracorporeal circuits with a volumetric flow rate, around 50 mL/minute to about 5 L/minute, *e.g.*, 50 mL/min, 100 mL/min, 150 mL/min, 200 mL/min, 250 mL/min, 300 mL/min, 350 mL/min, 400 mL/min, 500 mL/min, 550 mL/min, 600 mL/min, 650 mL/min, 700 mL/min, 750 mL/min, 800 mL/min, 850 mL/min, 900 mL/min, 950 mL/min, 1.0 L/min, 1.5 L/min, 2.0 L/min, 2.5 L/min, 3.0 L/min, 3.5 L/min, 4.0 L/min, 4.5 L/min, and 5 L/min. In some embodiments, the flow rate is preferably >150 mL/minute.

[0049] Highly porous adsorbent media, in contrast, requires much lower flow rates of less than 1 mL/minute to about less than 50 mL/minute. Additionally, the residence time on the

adsorption substrate (e.g., amount of time the adsorbate (e.g., bacteria) is in contact with the adsorbent media) needs to be much longer for a media requiring diffusive transport of adsorbates to the adsorbent site within the media, compared to a media using forced convection of adsorbates to the binding sites, which are not compatible with standard extracorporeal blood systems.

[0050] Typically, it is recognized that "residence time" on the adsorption column needs to be longer for a media requiring diffusive transport of adsorbates to the adsorbent site within the media, when compared to the lower residence time needed to convey an adsorbate to the binding site (on an essentially nonporous media) by forced convection. However, there are practical limits to the dimensions of a safe and effective adsorbent cartridge, column, filter, etc., especially with respect to the maximum hold-up volume of blood it can contain, and the flow velocity of blood or serum past the adsorption media. For this reason average flow rate through the adsorption device is considered to be a design variable.

[0051] Substrates that rely on forced convection transport are generally more suitable for high-flow rates, while substrates that rely on the much slower diffusion transport are much less effective when high flow rates and shorter residence times are required. For this reason, in an extracorporeal blood purification device, it is preferred that an adsorbate quickly diffuses through the pores within the adsorbent media. When blood is pumped through circuits fabricated from man-made materials, it is a general practice to employ relatively high blood flow rates in order to prevent stagnation and reduce the risk of clotting. On the other hand, extremely high flow rates may be avoided because they can expose blood cells to high shear rates and impingement damage that can rupture or otherwise damage blood cells. The present invention, therefore, provides a method and device for removing bacterial pathogens from blood using the preferred characteristics of convection transport and its desirable, more-rapid kinetics. This is achieved by passing/flowing blood over an essentially non-microporous substrate (e.g., a solid substrate), which is capable of binding the desired cytokine, pathogen or bacteria to remove them from the blood.

[0052] Adsorption media provided herein can be used in traditional extracorporeal blood circulation with flow rates >50 mL/min, and preferably between about 150 mL/minute to 5L/minute. If measured by linear flow velocity, $\geq$8 cm/min, preferably about $\geq$24 cm/min and

more preferably about 24-329 cm/min, or more. For example, the flow rate can be 25, 50, 75, 100, 125, 150,175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800 cm/min or more. Such high flow rates create short residence times within the adsorption column and convection transport dominates over Brownian diffusive transport. This is particularly important for binding larger particles such as viruses, bacteria and parasites and other proteins and pathogens that diffuse slowly.

[0053] The main adsorption sites available for removing bacterial pathogens lie at the surfaces within the interstices of the media bed, container or cartridge through which the blood flows or is delivered by forced convection. To treat blood, the interstitial channels need to be large enough to allow the transport of red blood cells, which are an average 6 microns in diameter. To allow a packed adsorption cartridge to be placed into an extracorporeal circuit with high blood flow rate, the interstitial channels can be several times larger than the diameter of red blood cells. This can prevent or substantially eliminate high shear rates that lead to hemolysis while simultaneously minimizing pressure drop in the blood that flows through the packed bed or cartridge. Additionally, the media is preferably rigid to minimize deformation that can clog the filter cartridge by compaction. Based on these preferences, an optimized rigid media balances interstitial channel size and total surface area, *e.g.*, for efficient removal of pathogens and/or cytokines in high-flow extracorporeal blood circuits.

[0054] The claimed methods are intended to be applied primarily in extracorporeal therapies or procedures, and also implantable devices.

[0055] Whole blood and blood serum from mammals can be used in the present invention. The amount of blood or blood serum that can be used in the claimed methods is not intended to be limited. It can range from less than 1 mL to above 1 L, up to and including the entire blood volume of a patient or subject when continuous recirculation back to the patient is employed. One or more passes through the adsorption bed may be used if needed. The blood may be human or animal blood.

[0056] In some embodiments, bacteria or pathogens in the sample, *e.g.*, whole blood or blood serum, is reduced by about 20% to about 90%, *e.g.*, about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 99.9%. In other embodiments, bacteria in the sample is reduced by about 20% to

about 40%, e.g., about 20%, 25%, 30%, 35%, or 40% or about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99.9% reduction of the bacteria or pathogen.

[0057] In some embodiments, the bacteria in the sample is a gram-negative bacteria, such as any bacteria that does not retain crystal violet dye. Non-limiting examples of a gram-negative bacteria are *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella*, *Shigella*, *Stenotrophomonas maltophilia*, *Moraxella*, *Borrelia*, *Burkolderia*, *Campylobacter*, *Chlamydia*, *Hemophilus*, *Helicobacter*, *Stenotrophomonas*, *Vibrio*, *Leginella*, other *Enterobacteriaceae*, and drug-resistant strains thereof. In other embodiments, the bacteria in the sample is a gram-positive bacteria, such as any bacteria that retains crystal violet dye. Non-limiting examples of a gram-positive bacteria are *Actinomyces*, *Bacillus*, *Enterococcus*, *Lactobacillus*, *Listeria monocytogenes*, *Mycobacterium*, *Nocardia*, *Propionibacteriaum*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptomyces*, *Streptococcus pneumoniae*, *Streptococcus pyrogenes*, *Streptococcus viridans*, *Enterococci*, *Clostridium difficile*, *Enterococcus* faecium, *Enterococcus* faecalis, and drug-resistant strains thereof.

[0058] In some embodiments, the methods provided herein are used to remove gram-negative bacteria from a whole blood or blood serum sample. In other embodiments, the methods are used to remove gram-positive bacteria from the sample. In yet other embodiments, the adsorption media described herein having a polysaccharide absorbant on its surface is used to remove bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterococcus faecium*, carbapenem-resistant *Escherichia coli*, carbapenem-resistant *Klebsiella pneumoniae*, and/ or extended spectrum beta-lactamase *Klebsiella pneumonia* from the sample.

[0059] In some embodiments, the absorption media having a neutrally charged hydrophilic surface is used to remove *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), and/or *Escherichia coli* from a whole blood or blood serum sample. In other embodiments, the adsorption media having a cationic surface (hydrophilic surface) is used to remove *Escherichia coli*, *Klebsiella pneumoniae*, carbapenem-resistant *Escherichia coli*, carbapenem-resistant *Klebsiella pneumoniae*, and extended spectrum beta-lactamase *Klebsiella pneumoniae*, *Enterococcus faecium*, *Acinetobacter baumannii*, and methicillin-resistant *Staphylococcus aureus* (MRSA) from the sample.

B. Adsorption Media

[0060] Various materials, in shape and composition, can be used as a substrate in the present invention. All suitable adsorbent substrates provide high surface area while promoting the conveyance of adsorbates to the adsorbent sites that bind them (primarily) by forced convective transport. Useful substrates for creating the adsorption media include non-porous rigid beads, particles, or packing, reticulated foams, a rigid monolithic bed (*e.g.* formed from sintered beads or particles), a column packed with woven or non-woven fabric, a column packed with a yarn or solid or hollow non-microporous monofilament fibers, a spiral wound cartridge formed from flat film or dense membrane, or a combination of media such as a mixed bead/fabric cartridge. In some embodiments, a suitable substrate for use in the present invention is one that is initially microporous, but becomes essentially non-porous when the surface is treated before, during or after the creation of adsorption sites.

[0061] One useful substrate is in the form of solid beads or particles. The beads can be made of materials that are sufficiently rigid to resist deformation or compaction under the encountered flow rates. In some embodiments, sufficient substrate rigidity is the absence of a significant increase in pressure drop across the adsorption bed during about one hour of flow of water or saline at typical clinical flow rates. For instance, a suitable substrate rigidity is a <10-50% increase in pressure drop relative to the initial pressure drop (*e.g.*, measured within the first minute of flow) when measured at a similar flow rate, *e.g.*, of saline.

[0062] The adsorbent substrate beads may be made from a number of different biocompatible materials, such as natural or synthetic polymers or non-polymeric materials including glasses, ceramics and metals, that are essentially free of leachable impurities. Some exemplary polymers including polyurethane, polymethylmethacrylate, polyethylene or co-polymers of ethylene and other monomers, polyethylene imine, polypropylene, and polyisobutylene. Examples of useful substrates include nonporous Ultra High Molecular Weight PolyEthylene (UHMWPE). Other suitable beads are polystyrene, high density and low density polyethylene, silica, polyurethane, and chitosan.

[0063] The substrate such as beads, fiber, yarn and the like can be prepared with a surface roughness or topography to increase the adsorption surface area. For example, it is possible to

increase the surface area by increasing the surface area to volume ratio. As is shown in FIG. 1A, an uneven and unulating surface produces more binding sites for the bacteria and pathogens. Typically a free form, shape, or geometry produces more surface area and is advantageous. FIG. 1A shows UHMWPE beads as received out of a reactor.

[0064] Methods for making beads are known in the art. For instance, suitable polyethylene beads and other polyolefin beads are produced directly during the synthesis process. In some instances, the beads are processed to the required size and shape. Other polymers may need to be ground or spray dried and classified, or otherwise processed to create beads of the desired size distribution and shape.

[0065] In some aspects, the adsorption media of the present invention provides a surface to attach a polysaccharide adsorbent that can bind a bacterial pathogen. In some embodiments, the adsorption media includes a solid substrate with a high surface area having at least one polysaccharide adsorbent on the surface thereof.

[0066] In other aspects, an adsorption media of the present invention provides a hydrophilic surface without a polysaccharide adsorbent ("a naked surface"). In some embodiments, the adsorption media includes a solid substrate with a high surface area and a hydrophilic cationic surface. In other embodiments, the adsorption media includes a solid substrate with a high surface area and a hydrophilic neutral surface.

[0067] The solid substrate can be made of, for example, but not limited to, polyethylene, polystyrene, polypropylene, polysulfone, polyacrylonitrile, polycarbonate, polyurethane, silica, latex, glass, cellulose, crosslinked agarose, chitin, chitosan, crosslinked dextran, crosslinked alginate, silicone, fluoropolymer, and other synthetic polymers. The solid substrate with a high surface area can be a plurality of adsorbent monolayers, filters, membranes, solid fibers, hollow fibers, particles, or beads. Optionally, the solid substrate can be present in other forms or shapes providing a large surface area.

[0068] In certain instances, the solid substrate is a plurality of rigid polymer beads such as polyethylene, polystyrene, polypropylene, polysulfone, polyacrylonitrile, polycarbonate, polyurethane, silica, latex, glass, cellulose, crosslinked agarose, chitin, chitosan, crosslinked

dextran, crosslinked alginate, silicone, fluoropolymer, and synthetic polymer beads. Preferably, the rigid polymer beads are polyethylene beads.

[0069] The size of the solid substrate can be selected according to the volume of the test sample used in the assay or other parameters. In some embodiments, each bead of the plurality of rigid polymer beads has an average outer diameter of about 1 μm to about 1 mm, *e.g.*, 1 μm, 2 μm, 3 μm, 4 μm, 5 μm, 6 μm, 7 μm, 8 μm, 9 μm, 10 μm, 15 μm, 20 μm, 25 μm, 30 μm, 35 μm, 45 μm, 55 μm, 60 μm, 65 μm, 70 μm, 75 μm, 80 μm, 85 μm, 90 μm, 95 μm, 100 μm, 200 μm, 300 μm, 400 μm, 500 μm, 600 μm, 700 μm, 800 μm, 900 μm, or 1 mm. In other embodiments, the each bead of the plurality of rigid polymer beads has an average diameter of about 10 μm to about 200 μm, *e.g.*, 10 μm, 15 μm, 20 μm, 25 μm, 30 μm, 35 μm, 45 μm, 55 μm, 60 μm, 65 μm, 70 μm, 75 μm, 80 μm, 85 μm, 90 μm, 95 μm, 100 μm, 105 μm,110 μm, 115 μm, 120 μm, 125 μm, 130 μm, 135 μm, 140 μm,145 μm, 150 μm,155 μm, 160 μm, 165 μm, 170 μm, 175 μm, 180 μm, 185 μm, 190 μm 195 μm, or 200 μm.

[0070] In some embodiments, useful beads have a size ranging from about 100 microns (μm) to 500 μm, or more in diameter, *e.g.*, 100 μm, 150 μm, 200 μm, 250 μm, 300 μm, 350 μm, 400 μm, 450 μm, 500 μm, or more, in diameter. The average size of the beads can be from about 150 μm to about 450 μm in diameter, *e.g.*, 150 μm, 200 μm, 250 μm, 300 μm, 350 μm, 400 μm, or 450 μm in diameter. For example, polyethylene beads from Polymer Technology Group (Berkeley, CA) having an average diameter of 300 μm are suitable for the present invention.

[0071] In some embodiments, the substrate is a barrier membrane, *e.g.*, a non-porous film. Alternatively, a microporous membrane may be rendered non-porous by filling the pores with essentially non-porous material, *e.g.*, a polymer. The membrane in the form of a sheet or a solid or hollow fiber may be arranged within a housing or a container.

[0072] The adsorption media can be in a vessel such as a column, cartridge, tube, centrifuge tube, bed, and the like, or any vessel wherein the cells of the blood that are not captured onto polysaccharide bound adsorption media can be removed without disturbing the bacterial pathogen attached to the media.

[0073] The substrate is typically provided packed within a housing or container, such as a column, that is designed to hold the substrate within the container and permit the blood or serum

to flow over the surface of the substrate or bed. The substrate may be arranged within the container to maximize the binding of the adsorbates to the absorbent sides of the substrate. The housing or container can have a macroporous surface structure that provides a large surface area to the blood or serum.

[0074] A column or other housing shape can be packed with either woven or non-woven heparinized fabric or the heparin, heparan sulphate or optional non-heparin adsorption sites may be attached, *e.g.* by covalent, ionic or other chemical or physical bonds, after the housing has been filled with the substrate media. By controlling the fiber denier and density of the fabric during weaving or knitting or during the creation of a non-woven web, the interstitial pore size can be controlled. Useful non-woven fabrics may be in the form of felts, melt-blown, or electrostatically spun webs, having a random orientation held together by entanglement of the fibers and/or adhesion or cohesion of intersecting fibers. Useful woven fabrics have a more defined and non-random structure.

[0075] A column or housing can be packed with fibers or yarns made from fibers. Polyethylene, and other fibers, can be drawn into thin hollow or solid monofilament fibers or multifilament yarns, which can be packed into cartridges in the same way that hollow fiber membranes, are installed within conventional hemodialysis cartridges or blood oxygenators. In the present invention, originally porous hollow fibers are rendered dense or non-porous before, during or after binding heparin or other adsorbents to the outer and/or inner surfaces. Dyneema Purity® from Royal DSM is a high-strength solid fiber made of UHMWPE. Ultra-high-molecular-weight polyethylene (UHMWPE, UHMW) is a subset of the thermoplastic polyethylene. Dyneema can be heparinized and packed into a cartridge to provide a high-surface area support for the removal of cytokines, bacteria and pathogens.

[0076] A spiral wound cartridge contains a thin film or membrane that is tightly wound together with optional spacer materials to prevent contact of adjacent surfaces. The membrane can be made from polymers such as polyurethane, polyethylene polypropylene, polysulfone, polycarbonate, PET, PBT, and the like.

[0077] As noted above, in certain instances, for use in the methods of the invention, the size of the channels or interstitial space between individual beads for extracorporeal blood filtration are optimized to prevent a high-pressure drop between the inlet and outlet of the cartridge, to permit

safe passage of the blood cells between the individual beads in a high flow environment, and to provide appropriate interstitial surface area for binding of the polysaccharide adsorbent to the cytokines or pathogens in the blood. For example, in a close packed bed of 300-micron, roughly spherical beads, an appropriate interstitial pore size is approximately 68 microns in diameter.

[0078] In some embodiments, the rigid beads of the adsorption media have an average diameter as is listed in Table 5. In some embodiments, the non-bead substrates of the adsorption media such as woven yarns or fibers have a macroscopic pore size as set forth in Table 6.

C. Methods for Making Adsorption Media

[0079] The surface of the solid substrate described herein can be functionalized to allow the covalent attachment of the polysaccharide adsorbent described herein. In some embodiments, the surface of the solid substrate has at least one chemical group, such as an amine group.

[0080] Polysaccharides such as heparin or heparan sulfate or other polysaccarides can be linked onto the surface of the adsorption media by covalent end-point attachment (*e.g.*, covalent attachment through the terminal residue of the heparin molecule). Covalent attachment as compared to non-covalent attachment advantageously provides better control of the orientation of the immobilized molecules and their surface density. In particular, the end-point attachment of these long chain carbohydrates provides a spacer function that leads to a higher concentration of accessible carbohydrate oligomers available for pathogen binding. In fact, certain pathogens attach to full-length heparin (*e.g.*, heparin with a mean molecular weight of more than 10 kDa) coated surfaces much more efficiently than to conventional surfaces coated with heparin fragments, as is generally employed in the art.

[0081] In some embodiments, the immobilized full-length heparin molecules have a mean molecular weight of more than 10 kDa. In other embodiments, the immobilized heparin molecules have a mean molecular weight of more than 15 kDa. In another embodiment, the immobilized heparin molecules have a mean molecular weight of more than 21 kDa. In yet another embodiment, the immobilized heparin molecules have a mean molecular weight of more than 30 kDa. Preferably, the immobilized heparin molecules have a mean molecular weight within the range of 15-25 kDa. The mean molecular weight may also be higher, such as in the range of 25-35 kDa.

[0082] In some embodiments, the surface concentration of the heparin adsorbent on the solid substrate is in the range of 1 μg/cm^2 to 20 μg/cm^2, *e.g.*, 1 μg/cm^2, 2 μg/cm^2, 3 μg/cm^2, 4 μg/cm^2, 5 μg/cm^2, 6 μg/cm^2, 7 μg/cm^2, 8 μg/cm^2, 9 μg/cm^2, 10 μg/cm^2, 11 μg/cm^2, 12 μg/cm^2, 13 μg/cm^2, 14 μg/cm^2, 15 μg/cm^2, 16 μg/cm^2, 17 μg/cm^2, 18 μg/cm^2, 19 μg/cm^2, and 20 μg/cm^2. In other embodiments, the surface concentration of the heparan adsorbent on the solid substrate is in the range of 5 μg/cm^2 to 15 μg/cm^2, *e.g.*, 5 μg/cm^2, 6 μg/cm^2, 7 μg/cm^2, 8 μg/cm^2, 9 μg/cm^2, 10 μg/cm^2, 11 μg/cm^2, 12 μg/cm^2, 13 μg/cm^2, 14 μg/cm^2, and 15 μg/cm^2.

[0083] The amount of polysaccharide adsorbent per gram substrate can vary. In one particular embodiment, if beads are used, the amount of polysaccharide, such as heparin per gram bead is determined by the number of layers used and also the size of the beads. The larger the bead, the less polysaccharide, such as heparin per gram of bead is achieved. One preferred amount is 2.0±0.5 mg heparin/g bead per the MBTH method (Larm *et al.*, *Biomater Med Devices Artif Organs*, 1983, 11:161-173 and Riesenfeld and Rosen, *Anal Biochem*, 1990, 188:383-389).

[0084] Covalent attachment of full-length heparin molecules to a surface can be achieved by the reaction of an aldehyde group of the heparin molecule with a primary amino group present on the surface of the adsorption media. An inherent property of all carbohydrates is that they have a hemiacetal in their reducing end. This acetal is in equilibrium with the aldehyde form and can form Schiffs bases with primary amines. These Schiffs bases may then be reduced to stable secondary amines. In some embodiments, full-length heparin is surface immobilized onto the solid substrate by covalent conjugation. In other embodiments, full-length heparin is covalently attached to said adsorption media via a stable secondary amino group.

[0085] In certain instances, various methods of making adsorbents and the adsorbents per se are disclosed in U.S. Patent No. 8,663,148 and U.S. Patent App. Publication Nos. US2009/0136586, US2010/0249689, US2011/0184377, and US2012/0305482, the disclosures of which are herein incorporated by reference in their entirety for all purposes.

[0086] In some embodiments, the adsorption media is hydrophilized prior to attachment of the polysaccharide, such as heparin, or other compounds. Methods for preparing the hydrophilic surface of the substrate include acid etching, plasma treating, and exposure to strong oxidizers. For instance, a polymeric surface such as a polyethylene (PE) bead can be etched with an oxidizing agent, such as potassium permanganate, ammonium peroxidisulfate and the like, to

introduce hydrophilic properties together with some reactive functional groups (*e.g.*, a sulfonyl group, a hydroxyl group, a carboxyl group, a carbonyl group, or carbon double bonds). The surface can be etched with plasma or corona. For example, PE beads can be etched with an potassium permanganate in sulfuric acid to produce beads with a hydrophilic surface containing hydroxyl groups and carbon double bonds.

D. Mixtures of Adsorption Media

[0087] In certain instances, the methods of the invention prepares the adsorption bed from a mixture of heparinized media which is antithrombogenic and another media which is inherently thrombogenic. By assembling an adsorption cartridge with both heparinized surfaces and, for example, hydrophilic surfaces (cationic or neutral surfaces), bacterial pathogens can all be safely removed from blood or other biological fluid. For example, the heparinized media can be from 1% to 99% of the adsorption bed and the and the inherently thrombogenic substrate can be from 99% to 1% of the adsorption bed.

[0088] In some embodiments of the present invention, the adsorption media provides an antithrombogenic surface that is in intimate contact with, or in close proximity to a thrombogenic surface. This adsorption media can prevent clinically significant thrombus formation that would otherwise occur if the inherently thrombogenic surface were used alone.

[0089] In the case of adsorption media in the form beads or particles, a preferred application of this invention is to blend the different adsorption media together before packing them into a cartridge or other housing. This provides intimate contact among the various surface chemistries on adjacent beads while permitting efficient manufacturing of adsorption cartridges or filters. A related approach is to layer the different media in a 'parfait-type' arrangement within the housing such that the blood contacts the different media in series or parallel flow. One arrangement of the different media within a cartridge is to position unblended antithrombogenic media at the entrance and/or the exit of the cartridge, with an optionally blended region containing the more thrombogenic media interposed between the entrance and exit regions.

[0090] In the case of media in fiber form, a mixed woven, knitted, or nonwoven structure can be prepared by methods well known in the textile industry to form fabric from the mixed fiber. Alternatively, a yarn can be prepared from finer multifilament yarn or monofilament made from

two or more fibers with different surface chemistries, as long as one fiber type contains a surface that actively prevents blood clotting on contact. The mixed-fiber yarn can then be used to prepare fabric for blood contact. Hollow fiber or solid fiber adsorption media can be blended and used to make cartridges that resemble hollow-fiber dialyzers or oxygenators. For membrane or film-type adsorption media of the type that is used in a spiral-wound adsorption cartridges, two or more surface chemistries may be used in close proximity to each other such that the blood must contact both surface chemistries (nearly) simultaneously. This can be done with a regular or random array of the various binding groups within the surface layer of the membrane film, or by forming a flow path for blood between two closely-spaced membrane films, one of which is antithrombogenic.

E. Extracorporeal Blood Filter

[0091] In certain aspects, methods provided herein can be used in a device comprising adsorption media for extracorporeal removal of pathogens from mammalian blood, *e.g.*, human blood. For instance, the device can be a conventional device for extracorporeal treatment of blood and serum from patients, *e.g.* a subject suffering from renal failure.

[0092] Local blood flow patterns in blood contacting medical devices for extracorporeal circulation are known to influence clot formation via shear activation and aggregation of platelets in stagnant zones. The device containing the adsorption media provided herein may, for example, have one or more of the following properties: a) a blood flow in the range of 150-5,000 ml/min, or if measured by linear flow velocity of ≥8 cm/min; b) low flow resistance; c) large surface area of substrate having carbohydrates immobilized thereto, *e.g.* about 0.1-1 m^2; d) a stable coating (*e.g.*, no clinically significant leakage of carbohydrate to the blood in contact therewith); e) proper hemodynamic properties in the device (*e.g.*, no stagnant zones); and f) optimal biocompatibility.

[0093] Non-limiting examples of a device for use according to the methods of the present invention include an extracorporeal membrane oxygenation (ECMO) device, a pediatric hemoflow dialyzer which is an extracorporeal blood filtration device for removing cytokine molecules or other extracorporeal device that can accommodate high flow rates.

[0094] The methods of the present invention can be employed either before or after other conventional treatments, such as administration of antibiotics.

[0095] In some embodiments, the methods are performed in a continuous loop such that, the sample, e.g., whole blood, is extracted from the body and processed according to the method provided herein, and then the resulting sample (e.g., sample containing a reduced amount of bacterial pathogen) is reintroduced into the body, thereby forming a loop comprising part of the bloodstream of the patient.

[0096] In other embodiments, the methods provided herein can be combined with other techniques to filter or treat mammalian blood. For example, a cartridge that is based on convection kinetics can then be used in series with conventional extracorporeal circuits such as cardiopulmonary bypass (CPB), hemodialysis, extracorporeal blood oxygenation and ozonation (EBOO), and the like.

[0097] The various aspects of the invention are further described in the following examples. These examples are not intended to be limiting. For instance, in the present examples heparin is used. However, other carbohydrates and polysaccharide adsorbents may be used alone or in addition to the heparin-coated substrates exemplified below.

III. EXAMPLES

[0098] The following examples are offered to illustrate, but not to limit, the claimed invention.

Example 1. Removal of Bacteria with Low or Undetectable Affinity for Heparan Sulfate

[0099] This example illustrates the use of heparin coated beads to remove bacterial pathogens with low affinity or undetectable affinity for heparan sulfate from whole blood.

[0100] It has been reported in the literature that over 50 different pathogens target heparan sulfate proteoglycans found on syndecans as an initial attachment site during their pathogenesis. Surprisingly, surface bound heparin can function as a surrogate to heparan sulfate binding organisms.

[0101] Our studies have shown that heparinized adsorption media can remove high concentration of *S. aureus* and MRSA from whole blood. Also, the study showed that the

bacteria attached to the heparinized surface were not killed, and thus did not release potential inflammatory toxins and their byproducts into the blood. Thus, the heparin-bound media can be used in an extracorporeal device to effectively and safely remove circulating bacteria including drug-resistant strains from infected blood.

[0102] This example tests both known heparan sulfate binding pathogens and pathogens either unknown or unexpected to bind to heparin. Additionally, it was discovered that hydrophilic controls, either cationic or neutrally charged, can function as an effective surface to bind pathogens. Neutrally charged surfaces in general were not as effective as heparinized surfaces in removing pathogens, but it is feasible that a pathogen reduction technology could be developed using generic hydrophilic surfaces. Hydrophilic cationic surfaces showed reasonable ability to remove pathogens as well.

[0103] This example illustrates that an adsorption media comprising a surface-bound heparin can be used to remove expected heparan sulfate binding pathogens such as, *S. aureus,* methicillin-resistant *S. aureus* (MRSA), *E. faecalis,* vancomycin-resistant *E. faecalis*, HSV-1 and HSV-2, and *Candida albicans*.

[0104] This example illustrates that an adsorption media comprising a surface-bound heparin can be used to remove low (*e.g.*, zero) affinity heparan sulfate-binding pathogens, such as, *E.coli,* carbapenem-resistant *E. coli*, *K. pneumoniae,* carbapenem-resistant *K. pneumoniae*, extended spectrum beta-lactamase *K. pneumoniae*, *E. faecium*, *A. baumannii*, and *S. pneumonia,* from blood.

[0105] In particular, an adsorption media comprising a neutral hydrophilic surface can remove, for example, *S. aureus,* methicillin-resistant *S. aureus* (MRSA), and *E.coli.* Also, an adsorption media comprising a cationic hydrophilic surface can remove, for example, *E.coli*, *K. pneumoniae,* carbapenem-resistant *K. pneumoniae,* extended spectrum beta-lactamase *K. pneumoniae*, *E. faecium*, *A. baumannii,* and methicillin-resistant *S. aureus* (MRSA).

[0106] *S. aureus* or methicillin-resistant S. aureus (MRSA) bacteremia exhibit a natural affinity towards heparin and heparin sulfate (HS). An affinity adsorption technology has been developed that relies on this natural mechanism to remove bacteria from blood. The primary ligand is end-point attached heparin, an analogue of heparan sulfate. Not only does the heparin

provide the mechanism of action to remove bacteria from whole blood, it also provides an anti-thrombogenic surface that enhances the safety of the extracorporeal circuit.

[0107] The targeting of carbohydrates and proteoglycans for initial attachment is a common mechanism of most pathogens. For instance, influenza viruses will bind to sialic acid, a carbohydrate found in many glycoproteins. Many gram negative bacteria have mannose binding adhesins located on the tips of fimbriae. Other carbohydrates that have shown to be targeted by bacteria include L-fucose, galactose, and various glucosamines or galactoamines. The common theme of pathogens binding to carbohydrates is the ubiquitous nature of the glycocalyx on cell surfaces.

[0108] The bacteria that have been targeted in this example include *E. coli*, *Klebsiella pneumoniae*, and their carbapenem-resistant strains, and also *P. aeruginosa*. There are many different adhesins reported for gram negative bacteria. The most studied are Fimbriae of Type 1, Type 3, Type P, and Type S and also outer membrane protein A (OmpA). Type 1 fimbriae and OmpA have been implicated in the attachment to endothelial cells. Type 1 fimbriae mediate attachment to mannose (mannose-sensitive) and are expressed in the majority of *Enterobacteriacea*. Other fimbriae have adhesins for different carbohydrates and are considered mannose-resistant. Typically, several types of fimbriae are expressed simultaneously.

[0109] In addition, it has been shown that mannose-sensitive adhesins are present on the bacterial cell surface even when fimbriae are not expressed. Type 1 fimbriae have been shown to interact with human brain microvascular endothelial cells suggesting that fimbriae can be expressed in blood. Drug resistant strains of *Klebsiella pneumoniae* express a higher concentration of both Type 1 and Type 3 fimbriae.

[0110] A heparinized surface to target removal of *S. aureus*, MRSA, *S. pneumoniae*, *E. faecalis*, *E. faecium*, herpes simplex virus, specific exotoxins, and other HS targeting pathogens was investigated. *In vitro* studies have confirmed the affinity of many of these pathogens and toxins for heparinized media.

[0111] The second adsorption media developed was a mannose functionalized surface to target gram negative bacteria, such as *E. coli*, *K. pneumoniae*, and *A. baumannii*. *In vitro* studies confirmed that mannose media can bind these pathogens. It was demonstrated that MRSA had

no affinity to the mannose media. However, the heparinized media was also very effective at removing these gram negative bacteria that were not expected to have a high affinity for heparin. These results were unexpected, and therefore it is not possible to predict based on literature alone which bacteria can be removed from blood by a heparinized surface.

Results

A. Results

[0112] The first report of successful removal of bacteria from whole blood was published in 2011 (Mattsby-Baltzer *et al.*, *J. Microbiol. Biotechnol.*, 2011, 21(6), 659-664). In this study, it was shown that a high concentrations of *S. aureus* and MRSA were removed from whole blood using the heparinized media. In addition, it was demonstrated using PCR that the bacteria were not killed when they attach to the heparinized surface and therefore did not release potential inflammatory toxins/byproducts into the bloodstream. The use of the heparinized media creates a very broad spectrum device that can safely remove circulating bacteria from blood, regardless of drug resistance.

[0113] The heparin adsorption media does not function by adding any detectable chemical substances to the treated blood or blood products. Instead it uses (non-leaching) covalently-bound, end-point-attached heparin as a ligand in a rapid adsorption process not limited by diffusion.

[0114] As discussed herein, *S. aureus* and MRSA can be removed from whole blood using the heparinized media. Several strains of *S. aureus* and MRSA were tested in this study. The results are shown in Table 1. *S. aureus* and several strains of MRSA were removed in high yield from whole blood. Depending on the strain, up to 85% of MRSA bacteria were removed by the heparinized media.

Table 1. Removal of S. Aureus and MRSA Strains From Whole Blood

***S. Aureus* and *MRSA* Strains tested**				
	SA1800T	**MRSA485**	**MRSA251**	**MRSA860**
% Removed in one pass	62%	85%	59%	70%

[0115] In an *in vitro* blood study , 85% of MRSA was removed by a single pass through the media (Table 2).

Table 2. Removal of both drug susceptible and drug resistant pathogens

Bacteria	% Reduction	Capacity (CFU/g)
Gram Positive Bacteria		
MRSA	91.57%	3.69E+05
S. pneumoniae	53.06%	1.73E+05
E. faecalis	99.04%	2.12E+06
E. faecalis(VRE)	91.25%	1.88E+06
E. faecium	56.38%	1.72E+06

[0116] The starting concentration of bacteria was 5 x 10^6 CFU/mL. In addition to binding *MRSA*, PCR analysis indicated that the heparinized surface was not bactericidal. This is an important finding that indicates cellular components of (dead) bacteria, which can be inflammatory and toxic to the recipient, are not released into the blood when bacteria attach to the media.

[0117] Additional studies were performed to test the affinitiy of various pathogens for the heparinized media. In these studies, 2.5 ml filter syringes were filled with heparinized media or control media to test the removal of various gram negative and gram positive bacteria. The bacteria were cultured using standard methods and diluted in defibrinated horse blood. The blood was then passed over the saline rinsed media a total of 3 times, and then plated for CFU counts. The targeted CFU/ml concentration was typical for antimicrobial testing and ranged between 10^5 and 10^6 CFU/ml.

[0118] A summary table reporting the removal of pathogens using the heparinized media is shown in Table 2.

B. Unexpected Results

[0119] Several pathogens reported in the literature with either little, no affinity, or unknown affinity to heparin or heparin sulfate were tested using the same protocols used for the heparin

bind pathogens. Table 3 lists these bacteria and the results. Surprisingly, many gram negative bacteria and their drug resistant strains were removed in high concentration from blood.

Table 3. Unexpected removal of gram negative bacteria using a heparinized surface

Gram Negative Bacteria	% Reduction	Capacity (CFU/g)
K. pneumoniae (CRE)	99.94%	4.66E+05
K. pneumoniae	36.57%	4.90E+05
E. coli (CRE)	99.93%	8.56E+05
E. coli	99.75%	2.04E+06
A. baumannii	79.13%	4.83E+05

Conclusion

[0120] The results show that heparinized media has an extremely high capacity to remove a broad spectrum of bacteria from blood. Unexpectedly, several bacteria with either no known affinity or had little affinity to heparin or heparin sulfate were also removed. Therefore, there is little predictability regarding the affinity that many pathogens may or may not have towards heparinized surface chemistry. The adsorption of several gram positive bacteria, including reported heparin binding pathogens, suggests that these pathogens bind specifically to the heparinized surface. Without being bound to any particular theory, it is believed that hydrophilic surfaces, such as neutral or cationic surfaces on the adsorption media, can be used to remove bacteria with no known affinity (or low affinity) to heparin or heparan sulfate. Alternatively, the binding of the above listed gram negative bacteria may be through interaction of specific sites or via non-specific binding. The surface topography of the adsorption media may be important to this binding.

Example 2. Adsorption media with a hydrophilic Surface

[0121] This example shows the adsorption media comprising a hydrophilic surface which can be used to removed bacteria from whole blood or serum.

[0122] The adsorption media described herein contains a surface topography that enables its binding to pathogens, such as those with no affinity or low affinity to heparin (FIG. 1A).

Without being bound by any particular theory, it is believed that a rough, uneven or ungulating surface may contribute to the affinity of the bacteria to the adsorption media.

[0123] FIG. 1B shows an image of a human blood smear for comparison. FIG. 2 shows a size comparison of bacteria, *e.g.*, *Staphylococcus aureus* and *Chlamydia*, and viruses, *e.g.*, pox virus, herpes virus, influenza virus, and picornavirus (polio).

Example 3. Blood filters for use in high linear flow rate extracorporeal therapies

[0124] This example provides an exemplary design of an extracorporeal filter cartridge that is used to accommodate high linear flow rates.

[0125] An extracorporeal blood filter can be designed to operate safely at specific flow rates used with common pump systems. If the pressure drop across a blood filter is too high, hemolysis can occur. Typically, dialysis systems operate with pressures below 34 kPa to avoid the risk of hemolysis.

[0126] For a cartridge filled with packed adsorbent media, the pressure drop across the cartridge depends on the flow rate, particle size, particle modulus, height of the packed media, and viscosity of blood. If a filter media is not sufficiently rigid, then compression of the media can occur with increased blood flow resulting in a reduced porosity that can lead to unsafe pressures.

[0127] The first variable to determine is the minimum particle size allowable for specific column heights and linear flow rates. Typical flow rates of dialysis systems are between 100 and 400 ml/min which equates to a linear flow rate of roughly 8 and 30 cm/min depending on the cartridge diameter. Typical volumetric flow rates of cardiopulmonnary bypass (CPB) and extracorporeal membrane oxygenators (ECMO) can be up to 5000 ml/min. Thus, depending on the cartridge width, the linear flow rate could be as high as 1000 cm/min. If a cartridge is made wider, the linear flow rate can be decreased to reduce pressure.

[0128] In determining the minimum particle size based on linear flow rate and particle size, it is necessary not to exceed pressures that can cause hemolysis. The Blake-Kozeny equation describes the pressure drop across packed media of rigid solids.

$$\Delta P = \mu * \left(\frac{Ko}{d_p^2}\right)\frac{(1-\varepsilon)^2}{\varepsilon^2} L * u$$

[0129] where μ is the viscosity of blood; K_o is a constant; d_p is the diameter of the particle; ϵ is the interstitial bed porosity or void volume; L is the height of the packed media; and u is the linear flow rate.

[0130] The equation can be solved for d_p

$$d_p = \sqrt{\frac{\mu * Ko}{\Delta P} * \frac{(1-\varepsilon)^2}{\varepsilon^2} L * u}$$

[0131] If 34 kPa is the maximum allowable pressure, then the following variables are used to determine particle size as a function of flow rate and column height.

μ =	4	cp	viscosity of blood
Ko =	150		constant
		(can range from 0.3 - 0.5 depending on packing	
ε =	0.36	efficiency)	
ΔP =	34	kPa	Maximum allowable pressure
	255	mmHg	
	4.9	PSI	

$$\frac{(1-\varepsilon)^2}{\varepsilon^2} = 8.78$$

$$\frac{\mu * Ko}{\Delta P} = 1.76E\text{-}05$$

[0132] The minimum bead diameter for a given linear velocity and column height are given in Table 4.

Table 4. Low Volumetric Flow Rates

bead diameter (microns)

	L (column height in cm)				
u (cm/min)	3	5	10	20	30
1	22	28	39	56	68
3	37	48	68	96	118
5	48	62	88	124	152
7	57	74	104	147	180

Table 4. Low Volumetric Flow Rates

bead diameter (microns)

	L (column height in cm)				
u (cm/min)	3	5	10	20	30
9	65	83	118	167	205
11	72	92	131	185	226
13	78	100	142	201	246
15	83	108	152	216	264
17	89	115	162	230	281
19	94	121	172	243	297
21	99	128	180	255	312
23	103	133	189	267	327
25	108	139	197	278	341
27	112	145	205	289	354
29	116	150	212	300	367
31	120	155	219	310	380

[0133] However, the size of blood cells can also be taken into account, as the effective pore size cannot be too small to block passage of blood cells. Macrophages are the largest cells in the blood and are about 21 microns, so it is important that these cells are allowed to pass through the filter media (FIG. 3).

[0134] The throat size represented by "a" in FIG. 3, i.e., the smallest opening between beads in a packed media, is described more fully below. The neck size can be calculated by the following equation.

$$a = d_p * \frac{2\sqrt{3}}{3} - 1$$

[0135] The minimum neck size must then be at least 21 microns. Therefore, the minimum bead size is:

$$d_{pmin} = \frac{a}{\frac{2\sqrt{3}}{3} - 1}$$

[0136] where d_{pmin} = 136 microns

[0137] Thus, the minimum size allowable is 136 microns. Table 5 represents useful linear flow rates and column heights for beads equal to or greater than 136 µm in diameter.

Table 5. Bead Size in Relation to Linear Flow and Column Height

bead diameter (microns)

	L (column height in cm)				
u (cm/min)	3	5	10	20	30
1	136	136	136	136	136
3	136	136	136	136	136
5	136	136	136	136	152
7	136	136	136	147	180
9	136	136	136	167	205
11	136	136	136	185	226
13	136	136	142	201	246
15	136	136	152	216	264
17	136	136	162	230	281
19	136	136	172	243	297
21	136	136	180	255	312
23	136	136	189	267	327
25	136	139	197	278	341
27	136	145	205	289	354
29	136	150	212	300	367
31	136	155	219	310	380

bead diameter (microns)

	L (column height in cm)				
u (cm/min)	3	5	10	20	30
1	136	136	136	136	136
76	188	243	343	485	594
151	265	342	484	684	838
226	324	418	592	837	1025
301	374	483	683	966	1183
376	418	540	763	1079	1322
451	458	591	836	1182	1448
526	494	638	903	1277	1564
601	529	682	965	1365	1671
676	561	724	1023	1447	1773
751	591	763	1079	1525	1868
826	620	800	1131	1600	1959
901	647	835	1181	1671	2046
976	674	870	1230	1739	2130
1051	699	902	1276	1805	2210
1126	723	934	1321	1868	2288

[0138] FIG. 4 represents a plot of Table 5. The plot shows the minimum bead size on the y axis, the linear flow rate on the x axis and the column height on the z axis. FIG. 4 has 6 distinct shades of grey as the bead size cut-off is 136 microns. Therefore, shades representing beads below that size are not represented. (e.g. 0-50 and 50-100).

[0139] The data was used to determine the minimum pore opening size of non-bead material such as woven yarns or fibers. The following table (Table 6) provides the corresponding minimum size of pore opening in relation to column height and linear flow rate.

Table 6. Macroscopic Pore Sizes for Non-Bead Material

Macroscopic Pore Size					
	L (column height in cm)				
u (cm/min)	3	5	10	20	30
1	21	21	21	21	21
3	21	21	21	21	21
5	21	21	21	21	24
7	21	21	21	23	28
9	21	21	21	26	32
11	21	21	21	29	35
13	21	21	22	31	38
15	21	21	24	33	41
17	21	21	25	36	43
19	21	21	27	38	46
21	21	21	28	39	48
23	21	21	29	41	51
25	21	22	30	43	53
27	21	22	32	45	55
29	21	23	33	46	57
31	21	24	34	48	59

Macroscopic Pore Size					
	L (column height in cm)				
u (cm/min)	3	5	10	20	30
1	21	21	21	21	21
76	29	38	53	75	92
151	41	53	75	106	130
226	50	65	92	129	159
301	58	75	106	149	183
376	65	83	118	167	205
451	71	91	129	183	224
526	76	99	140	197	242
601	82	106	149	211	259
676	87	112	158	224	274
751	91	118	167	236	289
826	96	124	175	247	303
901	100	129	183	258	317
976	104	135	190	269	329
1051	108	140	197	279	342
1126	112	144	204	289	354

[0140] If an adsorption media is compressible, the macroscopic pore size will decrease as a function of flow rate due to the shear stress of flowing blood. A compressible media can be "pre-compressed" to achieve the minimum pore size as calculated in Table 6 for a desired flow rate. For a loosely packed compressible media, the macroscopic pore size must not decrease below the values in the Table 6 under flow conditions, otherwise the pressure of the system will increase that could lead to hemolysis and macrophages would also be filtered out.

[0141] In addition to the determining particle size and/or macroscopic pore size, the diameter (*e.g.*, inner diameter) of the extracorporeal filter cartridge can determined. Table 7 provides useful cartridge diameters necessary to achieve the needed linear flow rate at a specific volumetric flow rate.

Table 7. Cartridge Diameters

	Diameter of Cartridge (cm)					
	Desired Volumetric Flow Rate (ml/min)					
u (cm/min)	50	100	150	300	500	1000
1	14.1	20.0	24.5	34.6	44.7	63.2
3	8.2	11.5	14.1	20.0	25.8	36.5
5	6.3	8.9	11.0	15.5	20.0	28.3
7	5.3	7.6	9.3	13.1	16.9	23.9
9	4.7	6.7	8.2	11.5	14.9	21.1
11	4.3	6.0	7.4	10.4	13.5	19.1
13	3.9	5.5	6.8	9.6	12.4	17.5
15	3.7	5.2	6.3	8.9	11.5	16.3
17	3.4	4.9	5.9	8.4	10.8	15.3
19	3.2	4.6	5.6	7.9	10.3	14.5
21	3.1	4.4	5.3	7.6	9.8	13.8
23	2.9	4.2	5.1	7.2	9.3	13.2
25	2.8	4.0	4.9	6.9	8.9	12.6
27	2.7	3.8	4.7	6.7	8.6	12.2
29	2.6	3.7	4.5	6.4	8.3	11.7
31	2.5	3.6	4.4	6.2	8.0	11.4

	Diameter of Cartridge (cm)					
	Desired Volumetric Flow Rate (ml/min)					
u (cm/min)	500	1000	2000	3000	4000	5000
1	44.7	63.2	89.4	109.5	126.5	141.4
76	5.1	7.3	10.3	12.6	14.5	16.2
151	3.6	5.1	7.3	8.9	10.3	11.5
226	3.0	4.2	5.9	7.3	8.4	9.4
301	2.6	3.6	5.2	6.3	7.3	8.2
376	2.3	3.3	4.6	5.6	6.5	7.3
451	2.1	3.0	4.2	5.2	6.0	6.7
526	1.9	2.8	3.9	4.8	5.5	6.2
601	1.8	2.6	3.6	4.5	5.2	5.8
676	1.7	2.4	3.4	4.2	4.9	5.4
751	1.6	2.3	3.3	4.0	4.6	5.2
826	1.6	2.2	3.1	3.8	4.4	4.9
901	1.5	2.1	3.0	3.6	4.2	4.7
976	1.4	2.0	2.9	3.5	4.0	4.5
1051	1.4	2.0	2.8	3.4	3.9	4.4
1126	1.3	1.9	2.7	3.3	3.8	4.2

[0142] Another factor to consider is the total blood volume used with an extracorporeal device. For instance, the total volume removed from the body during an extracorporeal circulation treatment is typically no more than 8-10% of the patient's blood. For an average adult, this equates to 500 ml of blood. A typical dialysis cartridge and tubing blood volume can range from 250-300 ml. If a dialysis cartridge is used in series with an adsorption cartridge, then the blood volume of the adsorption cartridge should be no more than 200 ml. The practical dimensions for an adsorption cartridge of the present invention is provided in Table 8.

Table 8. Blood Volume of Packed Cartridge (ml) - 0.36 void volume ratio

	Column Height (cm)				
Diameter	3	5	10	20	30
1	0.84834	1.4139	2.8278	5.6556	8.4834
5	21.2085	35.3475	70.695	141.39	212.085
10	84.834	141.39	282.78	565.56	848.34
15	190.8765	318.1275	636.255	1272.51	1908.765
20	339.336	565.56	1131.12	2262.24	3393.36

[0143] This example provides exemplary embodiments of the adsorption media and adsorption cartridge describe above. The adsorption media can be used in extracorporeal therapies with volumetric flow rate of up to 5000 ml/min and linear flow rates of up to 1000 cm/min.

Example 4. Blood filters for removal of Hepatitis C virus and Hepatitis B virus

[0144] This example provides an extracorporeal filter cartridge that is used to remove Hepatitis C virus and Hepatitis B virus. In this example, the adsorption media is mixed. The mixed media comprises a 70:30 ratio of heparinzed polyethylene beads : protein A fixed to a cellulose gel.

[0145] The heparinzed PE beads have covalent end-point attachment of nitrous acid degraded heparin onto aminated PE beads. The heparinized PE beads contain 2.6 mg heparin/g beads.

[0146] Covalent end-point attachment of nitrous acid degraded heparin onto aminated PE beads is prepared using 0.1 M acetate buffer pH 4.0 (100 ml) and nitrous acid degraded heparin (1.6 g). After shaking for 15 min, $NaBH_3CN$ (100 mg) dissolved in 0.1 M acetate buffer pH 4.0 (10 ml) is added. The reaction mixture is shaken for 24 h at room temperature and additional $NaBH_3CN$ (100 mg) dissolved in 0.1 M acetate buffer pH 4.0 (10 ml) is added, and shaking is continued for another 24 h at room temperature to produce the covalent end-point attachment of heparin.

[0147] In 0.5 mL of 0.05 M borate buffer (pH 10.0) is dissolved 4 mg of protein A (Sigma), and 0.01 N NaOH/water is added so as to bring the pH to 10 and make a total volume of 1.0 mL (protein A solution). This protein solution (total amount) is added to 1 mL of an epoxy-activated cellulose gel and the mixture is shaken at 37° C for 16 hours and washed with a sufficient amount of PBS (10 mM phosphate buffer supplemented with 150 mM sodium chloride) to provide GCL 2000m-Protein A.

[0148] The mixed adsorption media is used to remove Hepatitis C virus and Hepatitis B virus from blood.

[0149] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application

and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

WHAT IS CLAIMED IS:

1. An *ex vivo* method for removing bacteria from a sample taken from a subject who is suspected of being infected with bacteria, the method comprising:

contacting a sample taken from the subject with an adsorption media to allow the formation of an adhering complex, wherein the adhering complex comprises bacteria and the adsorption media; and

separating the sample from the adhering complex to produce the sample with a reduced amount of bacteria.

2. The method of claim 1, wherein the sample is selected from the group consisting of whole blood, serum and plasma.

3. The method of claim 2, wherein the sample is whole blood.

4. The method of claim 1, wherein the adsorption media is a solid substrate of high surface area having a hydrophilic surface that is free of a polysaccharide adsorbent.

5. The method of claim 4, wherein the solid substrate comprises a plurality of rigid polymer bead.

6. The method of claim 5, wherein the rigid polymer bead is a member selected from the group consisting of polyurethane, polymethylmethacrylate, polyethylene or co-polymers of ethylene and other monomers, polyethylene imine, polypropylene, and polyisobutylene.

7. The method of claim 4, wherein the solid substrate comprises one or a plurality of hollow fibers.

8. The method of claim 4, wherein the solid substrate comprises solid fibers or woven yarn made from solid fibers.

9. The method of claim 4, wherein the hydrophilic surface is a cationic surface.

10. The method of claim **1**, wherein the adsorption media has a high surface area as a result of surface or roughened topography.

11. The method of claim **4**, wherein the hydrophilic surface is a neutrally charged surface.

12. The method of claim **1**, wherein the bacteria in the sample is reduced by about 20% to about 99.9 %.

13. The method of claim **12**, wherein the bacteria in the sample is reduced by about 20% to about 40%.

14. The method of claim **1**, wherein the bacteria is a gram-negative bacteria.

15. The method of claim **1**, wherein the bacteria is a gram-positive bacteria.

16. The method of claim **1**, wherein the bacteria is selected from the group consisting of *Escherichia coli*, *Klebsiella pneumoniae*, carbapenem-resistant *Escherichia coli*, carbapenem-resistant *Klebsiella pneumoniae,* and extended spectrum beta-lactamase *Klebsiella pneumoniae, Enterococcus* faecium, *Acinetobacter baumannii*, and methicillin-resistant *Staphylococcus aureus* (MRSA).

17. The method of claim **1**, wherein the bacteria is selected from the group consisting of *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Escherichia coli*.

18. The method of claim **1**, wherein the sample has a linear flow rate of about 8 cm/min to about 1000 cm/min.

19. The method of claim **1**, wherein the sample has a volumetric flow rate of about 50 ml/min to about 5 L/min.

20. An *ex vivo* method for removing bacteria from a sample taken from a subject who is suspected of being infected with bacteria, wherein the bacteria are known to have a low affinity or no affinity for heparan sulfate, the method comprising:

contacting a sample taken from the subject with an adsorption media to allow the formation of an adhering complex, wherein the adsorption media is a solid substrate of high surface area having at least one polysaccharide adsorbent on the surface thereof; and

separating the sample from the adhering complex to produce the sample with a reduced amount of bacteria.

21. The method of claim **20**, wherein the sample is selected from the group consisting of whole blood, serum and plasma.

22. The method of claim **21**, wherein the sample is whole blood.

23. The method of claim **20**, wherein the solid substrate comprises a plurality of rigid polymer bead.

24. The method of claim **20**, wherein the adhering complex comprises bacteria and the adsorption media;

25. The method of claim **23**, wherein the rigid polymer bead is a member selected from the group consisting of polyurethane, polymethylmethacrylate, polyethylene or co-polymers of ethylene and other monomers, polyethylene imine, polypropylene, and polyisobutylene.

26. The method of claim **20**, wherein the solid substrate comprises one or a plurality of hollow fibers.

27. The method of claim **20**, wherein the at least polysaccharide absorbent is a member selected from the group consisting of heparin, heparan sulfate, hyaluronic acid, sialic acid, carbohydrates with mannose sequences, and chitosan.

28. The method of claim **27**, wherein the at least polysaccharide absorbent is heparin or heparan sulfate.

29. The method of claim **28**, wherein the at least polysaccharide absorbent is heparin.

30. The method of claim **29**, wherein the beads are coated with about 0.27 mg to about 10 mg heparin per gram of bead.

31. The method of claim **30**, wherein the bead is coated with 2 ±0.5 mg heparin per gram of bead.

32. The method of claim **20**, wherein the bacteria in the sample is reduced by about 20% to about 99.9%.

33. The method of claim **20**, wherein the bacteria in the sample is reduced by about 20% to about 40%.

34. The method of claim **20**, wherein the bacteria in the sample fail an *in vitro* heparin binding assay.

35. The method of claim **16**, wherein the bacteria is a gram-negative bacteria.

36. The method of claim **16**, wherein the bacteria is a gram-positive bacteria.

37. The method of claim **20**, wherein the bacteria is selected from the group consisting of *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterococcus faecium*, carbapenem-resistant *Escherichia coli*, carbapenem-resistant *Klebsiella pneumoniae*, and extended spectrum beta-lactamase *Klebsiella pneumoniae*.

38. The method of claim **20**, wherein the sample has a linear flow rate of about 8 cm/min to about 1000 cm/min.

39. The method of claim **20**, wherein the sample has a volumetric flow rate of about 50 ml/min to about 5 L/min.

40. An *ex vivo* method for removing bacteria from a sample taken from a subject undergoing dialysis, the method comprising:

contacting a sample taken from the subject with an adsorption cartridge comprising adsorption media, wherein the adsorption cartridge is in series with a dialysis cartridge to allow the formation of an adhering complex; and

separating the sample from the adhering complex to produce the sample with a reduced amount of bacteria.

41. The method of claim **40**, wherein the sample has a total blood volume of less than 200 ml.

42. The method of claim **40**, wherein the adsorption cartridge has a column height between 1 cm-50 cm.

43. The method of claim **40**, wherein the adsorption cartridge has a column diameter between 1 cm-50 cm.

44. The method of claim **40**, wherein the adsorption cartridge is proximal to the subject compared to the dialysis cartridge.

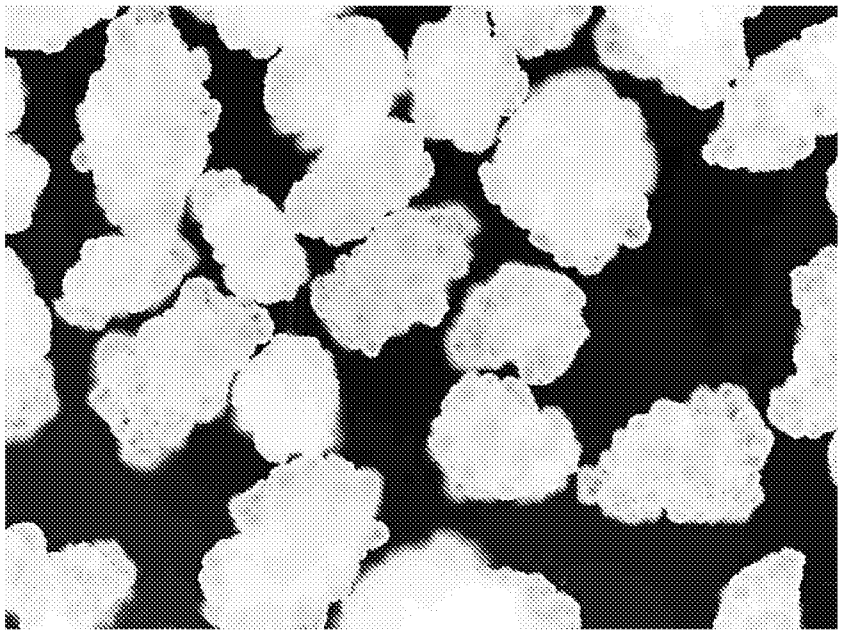
45. The method of claim **40**, wherein the adsorption cartridge is distal to the subject compared to the dialysis cartridge.

46. The method of claim **40**, wherein the adhering complex comprises bacteria and adsorption media.

47. The method of claim **40**, wherein the sample has a linear flow rate of about 8 cm/min to about 1000 cm/min.

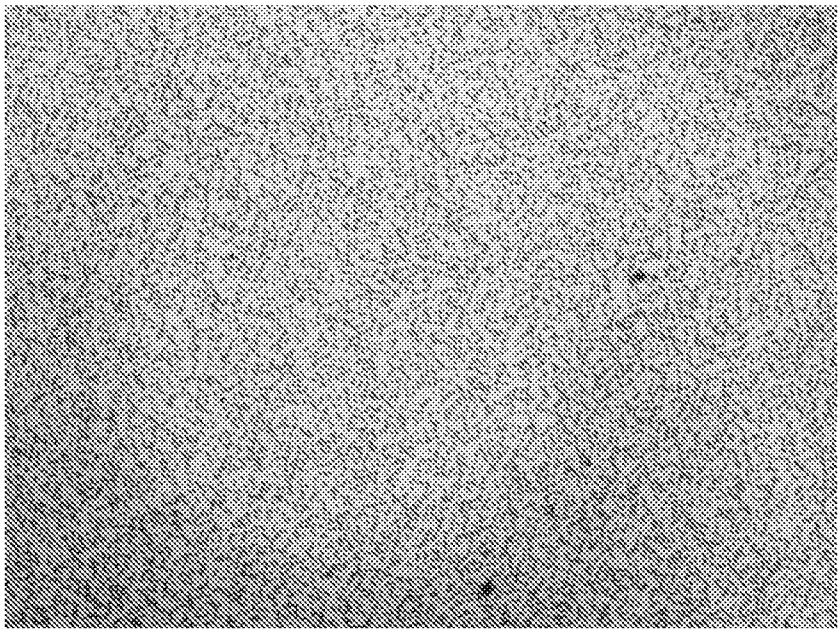
48. The method of claim **40**, wherein the sample has a volumetric flow rate of about 50 ml/min to about 5 L/min.

FIG. 1A



100x magnification

FIG. 1B



100x magnification

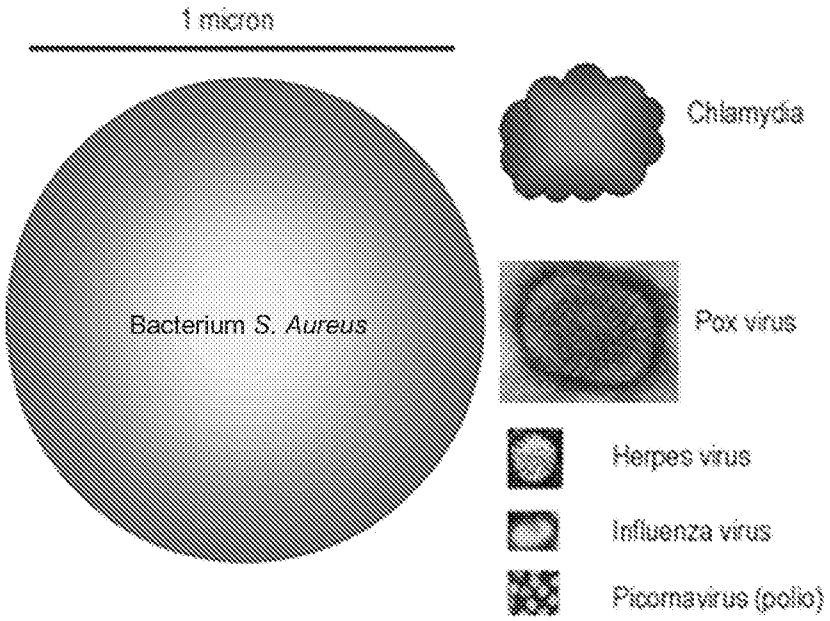
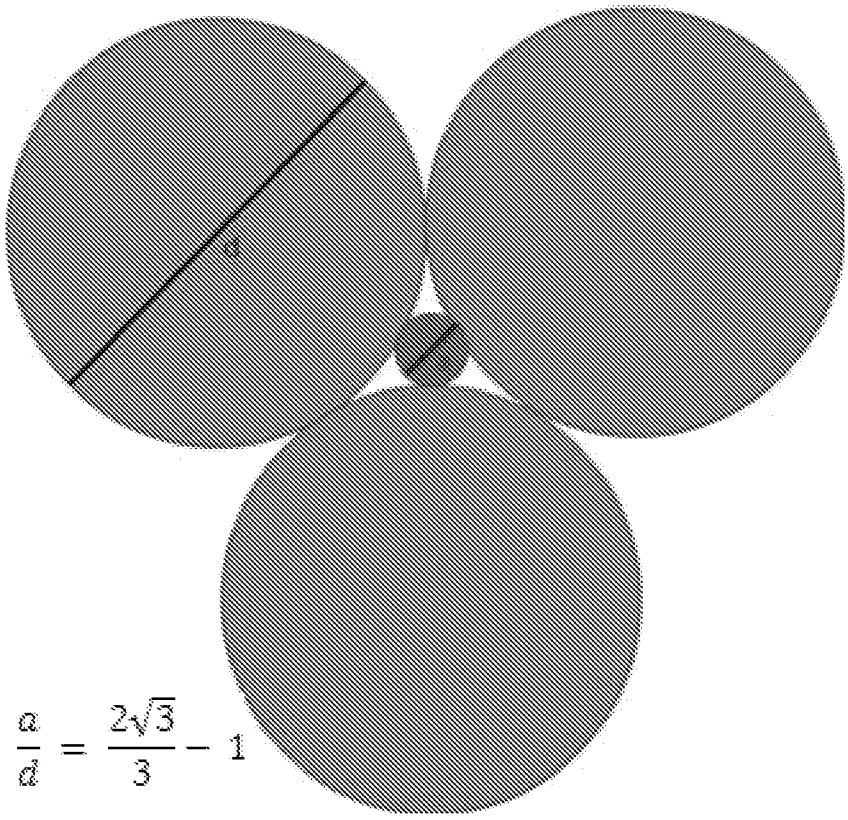

1 micron
Bacterium S. Aureus
Chlamydia
Pox virus
Herpes virus
Influenza virus
Picornavirus (polio)

FIG. 2



$$\frac{a}{d} = \frac{2\sqrt{3}}{3} - 1$$

FIG. 3

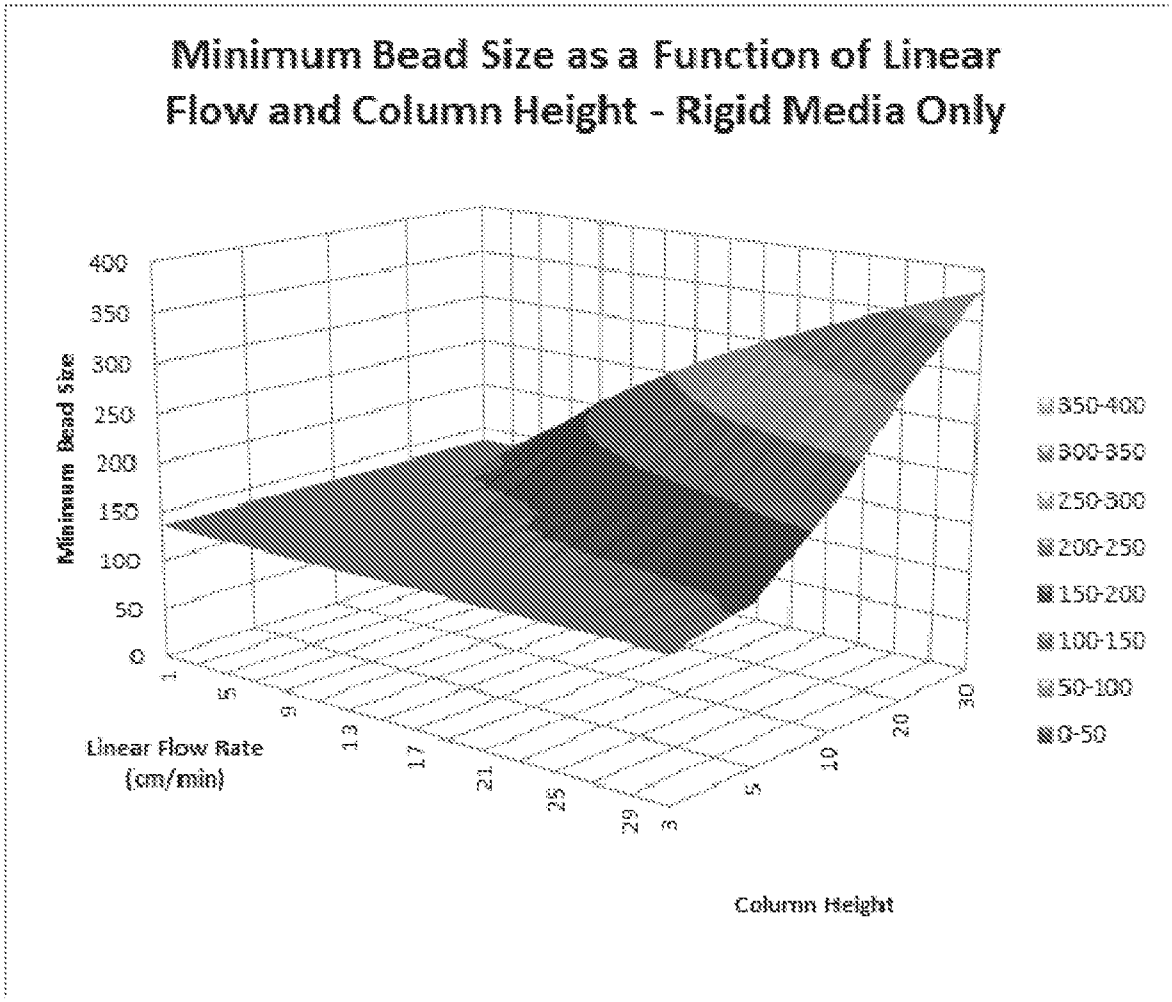

Minimum Bead Size as a Function of Linear Flow and Column Height - Rigid Media Only
Minimum Bead Size
400
350
300
250
200
150
100
50
0
Linear Flow Rate (cm/min)
1
5
9
13
17
21
25
29
Column Height
3
5
10
20
30
350-400
300-350
250-300
200-250
150-200
100-150
50-100
0-50

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/26340

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61M 1/36 (2015.01)
CPC - A61M 1/36
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(8): A61K 31/70; A61M 1/36, 5/00; B01D 61/00 (2015.01)
CPC: A61K 31/60, 31/722, 31/727; A61M 1/36

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Dialog ProQuest; IP.com; Google; Google Scholar; 'ex vivo,' remove, bacteria, infection, rigid, bead, separate, sample

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2011/0184377 A1 (WARD, RS et al.) July 28, 2011; abstract; paragraphs [0010], [0016], [0018], [0022], [0023], [0025], [0031], [0033], [0045], [0047], [0051], [0052], [0067], [0069], [0074], [0079], [0080], [0109]; Claim 26	1-10, 14-31, 34-41, 46-48 ----- 11-13, 32, 33, 42-45
Y	US 5476509 A (KEOGH, JR et al.) December 19, 1995; column 1, lines 66-67 to column 2, lines 1-6; column 12, lines 29-31; column 12, lines 40-41	11
Y	US 2007/0190050 A1 (DAVIDNER, AA et al.) August 16, 2007; abstract; paragraph [0087]	12, 13, 32, 33
Y	US 2013/0131423 A1 (WANG, T et al.) May 23, 2013; abstract; paragraph [0130]	42, 43
Y	US 2005/0098500 A1 (COLLINS, G et al.) May 12, 2005; abstract; figure 1, paragraph [0035]	44, 45

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent 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
07 July 2015 (07.07.2015)	28 JUL 2015
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)