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(54) **Title**: TREATING INFECTION BY A PLATELET-TARGETING MICROBE USING NANOPARTICLES

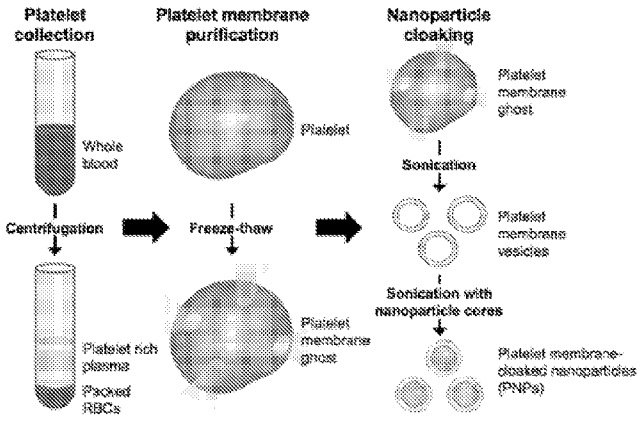


Figure 4B

(57) **Abstract**: The present invention relates to prevention and/or treatment of infection by a platelet-targeting microbe in a subject. The present invention provides for methods, combinations and pharmaceutical compositions for preventing and/or treating (and/or related uses) infection by a platelet-targeting microbe in a subject, using, *inter alia*, an effective amount of a nanoparticle comprising a) an inner core comprising a non-cellular material, b) an outer surface comprising a cellular membrane derived from a platelet; and optionally c) an agent for preventing said infection, treating said infection, diagnosing said infection, prognosing said infection and/or monitoring prevention or treatment of said infection. Exemplary platelet-targeting infections include infections by a bacterium, a virus, a fungus and/or a parasite.

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TREATING INFECTION BY A PLATELET-TARGETING MICROBE USING NANOPARTICLES

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0001] This invention was made with government support under Grant No. DK095168 awarded by the National Institutes of Health and under Grant No. HDTRA1-14-1-0064 awarded by the Defense Threat Reduction Agency. The government has certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims priority to U.S. Provisional Application Serial No. 62/181,793, filed June 20, 2015, the entire contents of which is incorporated by reference herewith.

FIELD OF THE INVENTION

[0003] The present invention relates to prevention and/or treatment of infection by a platelet-targeting microbe in a subject. The present invention provides for methods, combinations and pharmaceutical compositions for preventing and/or treating (and/or related uses) infection by a platelet-targeting microbe in a subject, using, *inter alia*, an effective amount of a nanoparticle comprising a) an inner core comprising a non-cellular material, b) an outer surface comprising a cellular membrane derived from a platelet; and optionally c) an agent for preventing said infection, treating said infection, diagnosing said infection, prognosing said infection and/or monitoring prevention or treatment of said infection. Exemplary platelet-targeting infections include infections by a bacterium, a virus, a fungus and/or a parasite.

BACKGROUND OF THE INVENTION

[0004] Development of functional nanoparticles can be encumbered by unanticipated material properties and biological events, which can negatively impact nanoparticle effectiveness in complex, physiologically relevant systems[1-3]. Despite the advances in bottom-up nanoengineering and surface chemistry, reductionist functionalization

approaches remain inadequate in replicating the complex interfaces present in nature and cannot avoid exposure of foreign materials.

[0005] New methods and compositions for preventing and/or treating (and/or related uses) infection by a platelet-targeting microbe in a subject are needed. The present disclosure addresses this and the related needs in the art.

SUMMARY OF THE INVENTION

[0006] In one aspect, the present invention provides for a method for preventing and/or treating infection by a platelet-targeting microbe in a subject, which method comprises administering, to a subject in need, or to cells of said subject, an effective amount of a nanoparticle comprising a) an inner core comprising a non-cellular material, b) an outer surface comprising a cellular membrane derived from a platelet; and optionally c) an agent for preventing said infection, treating said infection, diagnosing said infection, prognosing said infection and/or monitoring prevention or treatment of said infection.

[0007] In another aspect, the present invention is directed to use of an effective amount of a nanoparticle for the manufacture of a medicament for preventing and/or treating infection by a platelet-targeting microbe in a subject, wherein said nanoparticle comprises: a) an inner core comprising a non-cellular material, b) an outer surface comprising a cellular membrane derived from a platelet; and optionally c) an agent for preventing said infection, treating said infection, diagnosing said infection, prognosing said infection and/or monitoring prevention or treatment of said infection.

[0008] In still another aspect, the present invention provides for a combination for preventing and/or treating infection by a platelet-targeting microbe in a subject, which combination comprises an effective amount of a nanoparticle and an effective amount of a second prophylactic or therapeutic agent for preventing and/or treating infection by a platelet-targeting microbe in a subject, wherein said nanoparticle comprises: a) an inner core comprising a non-cellular material, b) an outer surface comprising a cellular membrane derived from a platelet; and optionally c) an agent for preventing said infection, treating said infection, diagnosing said infection, prognosing said infection and/or monitoring prevention or treatment of said infection.

[0009] In some aspects, the prevent disclosure relates to U.S. Application Serial No. 13/827,906, filed March 14, 2013, International Application No. PCT/US2012/039411, filed May 24, 2012 and published as WO 2013/052167 A2 and U.S. provisional application Serial No. 61/492,626, filed June 2, 2011. The contents of the above applications are incorporated by reference in their entireties.

BRIEF DESCRIPTION OF THE DRAWINGS

[00010] Figures 1A-1I: Preparation and characterization of PNPs. Figure 1A shows a physicochemical characterization of platelets, platelet membrane-derived vesicles (platelet vesicles), bare PLGA nanoparticles (bare NPs), and PNPs (n=3). Figure 1B shows a TEM images of bare NPs (left) and PNPs (right) negatively stained with uranyl acetate. Scale bar = 100 nm. Figure 1C shows a dynamic light scattering characterization of particle diameter of bare NPs and PNPs in water and in 1X PBS (n=3). Figure 1D shows a membrane protein translocation onto PNPs resolved using western blotting. Figure 1E shows a TEM image of PNPs negatively stained with vanadium, primary stained with extracellular-domain-specific anti-CD47, and secondary stained by an immunogold conjugate. Scale bar = 40 nm. Figures 1F-1H show platelet-activating contents including (Figure 1F) thrombin, (Figure 1G) ADP, and (Figure 1H) thromboxane in platelets, platelet vesicles, and PNPs were quantified (n=3). Figure 1I shows platelet aggregation assay in which citrate-stabilized platelet rich plasma (PRP) was mixed with PBS, PNPs, or thrombin followed by spectroscopic examination of solution turbidity. A decrease in solution turbidity indicates platelet aggregation and sedimentation. All bars represent means ± SD.

[00011] Figures 2A-2F: Collagen binding and immunocompatibility. Figure 2A shows fluorescence quantification of nanoparticle retention on collagen-coated and non-coated plates (n=6). Figure 2B shows a localization of PNPs (stained in red) on collagen-coated tissue culture slides seeded with HUVECs (nuclei stained in blue). Cellular periphery is outlined based on cytosolic staining. Scale bar = 10 μm. Figure 2C shows a pseudocolored SEM image of the extracellular matrix of a decellularized human umbilical cord artery following PNP incubation. Particles of approximately 100 nm can be observed on the substrate (colored in orange). Scale bar = 500 nm. Figure 2D shows a flow cytometric analysis of nanoparticle uptake by human THP-1 macrophage-like cells (n=3). Figure 2E

shows a classical complement activation as measured by C4d split products and Figure 2F shows an alternative complement activation as measured by Bb split products for bare NPs, platelet vesicles, and PNPs in autologous human plasma (n=4). Zymosan and untreated plasma are used as positive and negative controls respectively. All bars represent means ± SD. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

[00012] Figures 3A-3I: Binding to platelet-adhering pathogens. Figure 3A shows SEM images of methicillin-resistant *Staphylococcus aureus* (MRSA252 strain) following incubation with PBS (top left), bare NPs (top right), RBCNPs (bottom left), and PNPs (bottom right). Scale bar = 1 μm. Figure 3B shows normalized fluorescence intensity of DiD-loaded nanoformulations retained on MRSA252 based on flow cytometric analysis. Bars represent means ± SD (n=3). Figure 3C shows *in vitro* antimicrobial efficacy of free vancomycin, vancomycin-loaded RBCNPs (RBCNP-Vanc), and vancomycin-loaded PNPs (PNP-Vanc) upon 5 min of incubation with MRSA252 followed by 5 h of culturing. Bars represent means ± SD (n=3). Figures 3D-3I show *in vivo* antimicrobial efficacy of free vancomycin at 10 mg kg^{-1} (Vanc-10), RBCNP-Vanc-10, and PNP-Vanc-10, and free vancomycin at 6 times the dosing (Vanc-60, 60 mg kg^{-1}) was examined in a mouse model of systemic infection with MRSA252. Following 3 days of treatments, bacterial loads in different organs including (Figure 3D) blood, (Figure 3E) heart, (Figure 3F) lung, (Figure 3G) liver, (Figure 3H) spleen, and (Figure 3I) kidney were quantified. Bars represent means ± SEM (n=12 to 14). *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001.

[00013] Figures4A-4B: Figure 4A shows a schematic depicting the preparation of PNPs and their resulting functionalities. Poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles are enclosed entirely in plasma membrane derived from human platelets. The resulting particles possess platelet-mimicking properties for immunocompatibility, subendothelium binding, and pathogen adhesion. Figure4B shows a schematic depicting the process of preparing platelet membrane-cloaked nanoparticles (PNPs).

[00014] Figures 5A-5H show PNP preparation and storage. Figure 5A shows isolation of platelet rich plasma (PRP) was achieved via centrifugation at 100 × g. PRP was collected from the top layer (yellow) separated from the red blood cells (red, bottom layer). Figure 5B shows collected human platelets under light microscopy, which possess a distinctive morphology from Figure 5C red blood cells. Scale bars = 10 μm. Figure 5D

shows transmission electron micrographs of platelet membrane vesicles and Figure 5E shows PNPs, both of which were negatively stained with 1% uranyl acetate. Scale bars = 200 nm. Figure 5F shows dynamic light scattering measurements of PNPs in 10% sucrose show that the particles retain their size and stability following a freeze-thaw cycle and re-suspension upon lyophilization (n=3). Bars represent means ± SD. Figure 5G shows transmission electron micrograph shows retentions of PNPs' core-shell structure following a freeze-thaw cycle in 10% sucrose. Scale bar = 100 nm. Figure 5H shows transmission electron micrograph shows retentions of PNPs' core-shell structure upon resuspension following lyophilization in 10% sucrose. Scale bar = 100 nm.

[00015] Figure 6 shows overall protein content on PNPs resolved by western blotting. Primary platelet membrane protein/protein subunits such as CD47, CD55, CD59, αIIbβ3, α2, α5, α6, β1, GPIbα, GPIV, GPV, GPVI, GPIX, and CLEC-2 were monitored in platelet rich plasma, platelets, platelet vesicles, and PNPs. Each sample was normalized to equivalent overall protein content prior to western blotting.

[00016] Figures 7A-7D show platelet membrane sidedness on PNPs. Figure 7A shows transmission electron micrograph of PNPs primary stained with anti-CD47 (intracellular), secondary stained with immunogold, and negatively stained with 2% vanadium. The immunogold staining revealed presence of intracellular CD47 domains on collapsed platelet membrane vesicles but not on PNPs. Figure 7B shows Transmission electron micrograph of PNPs primary stained with anti-CD47 (extracellular), secondary stained with immunogold, and negatively stained with 2% vanadium. PNPs are shown to display extracellular CD47 domains. All scale bars = 100 nm. Figure 7C shows 2 μm polystyrene beads were functionalized with anti-CD47 against the protein's extracellular domain, anti-CD47 against the protein's intracellular domain, or a sham antibody. Flow cytometric analysis of the different beads following DiD-loaded PNP incubation shows the highest particle retention to beads functionalized with anti-CD47 against the protein's extracellular domain. Figure 7D shows Normalized fluorescence intensity of PNP retention to the different antibody-functionalized beads. Bars represent means ± SEM.

[00017] Figures 8A-8I shows collagen-coated tissue culture slides seeded with human umbilical vein endothelial cells (HUVECs) were incubated with PNP solution for 30 sec. Fluorescence microscopy samples demonstrate differential PNP adherence to exposed

collagen versus covered endothelial surfaces. Figures 8A-8C shows representative fluorescence images visualizing DiD-loaded PNPs (red grey scales), cellular cytosol (green grey scales), and cellular nuclei (blue grey scales). Figure 8D-8F shows images showing only the red and blue channels in order to highlight the differential localization of PNPs. Scale bar = 10 μm. Figure 8G shows fluorescence quantification of PNP per unit area on collagen and endothelial surfaces. Bars represent means ± SD (n=10). Figures 8H-8I shows PNP adherence to arterial extracellular matrix (ECM) as visualized by SEM. Figure 8H shows SEM images of the ECM of a decellularized human umbilical cord artery. Left: Scale bar = 1 μm; Right: Scale bar = 500 nm. Figure 8I shows SEM images of the ECM of a decellularized human umbilical cord artery following PNP incubation. Left: Scale bar = 1 μm; Right: Scale bar = 500 nm.

[00018] Figures 9A-9C show pharmacokinetics, biodistribution, and safety of PNPs. Figure 9A shows DiD-loaded PNPs were injected intravenously through the tail vein of Sprague-Dawley rats. At various time points blood was withdrawn via tail vein blood sampling for fluorescence quantification to evaluate the systemic circulation lifetime of the nanoparticles (n=6). Figure 9B shows biodistribution of the PNP nanoparticles in balloon-denuded Sprague-Dawley rats at 2 h and 48 h following intravenous nanoparticle administration through the tail vein (n=6). Figure 9C shows comprehensive metabolic panel of rats following injections with human-derived PNPs and PBS (n=6). The rats received intravenous injections of PNPs and PBS on day 0 and day 5, and the blood test conducted on day 10 did not reveal significant changes between the two groups, indicating normal liver and kidney functions following the PNP administration. All bars and markers represent means ± SD.

[00019] Figures 10A-10C show PNP adherence to MRSA252 bacteria. Figure 10A shows flow cytometric analysis of MRSA252 bacteria following incubation with different DiD-loaded nanoformulations. Figure 10B shows Pellets of MRSA252 following incubation with DiD-loaded RBCNPs (left) and DiD-loaded PNPs (right) show differential retention of nanoformulation with MRSA252 upon pelleting of the bacteria. Figure 10C shows a pseudocolored SEM image of PNPs binding to MRSA252 under high magnification (MRSA colored in gold, PNP colored in orange). Scale bar = 400 nm.

DETAILED DESCRIPTION OF THE INVENTION

[00020] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of nanotechnology, nano-engineering, molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, immunology, and pharmacology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, 2nd ed. (Sambrook et al., 1989); Oligonucleotide Synthesis (M. J. Gait, ed., 1984); Animal Cell Culture (R. I. Freshney, ed., 1987); Methods in Enzymology (Academic Press, Inc.); Current Protocols in Molecular Biology (F. M. Ausubel et al., eds., 1987, and periodic updates); PCR: The Polymerase Chain Reaction (Mullis et al., eds., 1994); and Remington, The Science and Practice of Pharmacy, 20th ed., (Lippincott, Williams & Wilkins 2003).

[00021] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. All patents, applications, published applications and other publications referred to herein are incorporated by reference in their entireties. If a definition set forth in this section is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth in this section prevails over the definition that is incorporated herein by reference.

A. Definitions

[00022] To facilitate understanding of the invention, a number of terms and abbreviations as used herein are defined below as follows:

[00023] When introducing elements of the present invention or the preferred embodiment(s) thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements.

[00024] The term "and/or" when used in a list of two or more items, means that any one of the listed items can be employed by itself or in combination with any one or more of the listed items. For example, the expression "A and/or B" is intended to mean either or both of A and B, *i.e.* A alone, B alone or A and B in combination. The expression "A, B and/or C" is intended to mean A alone, B alone, C alone, A and B in combination, A and C in combination, B and C in combination or A, B, and C in combination.

[00025] Cellular Membrane: The term "cellular membrane" as used herein refers to a biological membrane enclosing or separating structure acting as a selective barrier, within or around a cell or an emergent viral particle. The cellular membrane is selectively permeable to ions and organic molecules and controls the movement of substances in and out of cells. The cellular membrane comprises a phospholipid uni- or bilayer, and optionally associated proteins and carbohydrates. As used herein, the cellular membrane refers to a membrane obtained from a naturally occurring biological membrane of a cell or cellular organelles, or one derived therefrom. As used herein, the term "naturally occurring" refers to one existing in nature. As used herein, the term "derived therefrom" refers to any subsequent modification of the natural membrane, such as isolating the cellular membrane, creating portions or fragments of the membrane, removing and/or adding certain components, such as lipid, protein or carbohydrates, from or into the membrane taken from a cell or a cellular organelle. A membrane can be derived from a naturally occurring membrane by any suitable methods. For example, a membrane can be prepared or isolated from a cell or a virus and the prepared or isolated membrane can be combined with other substances or materials to form a derived membrane. In another example, a cell or virus can be recombinantly engineered to produce "non-natural" substances that are incorporated into its membrane *in vivo*, and the cellular or viral membrane can be prepared or isolated from the cell or the virus to form a derived membrane.

[00026] In various embodiments, the cellular membrane covering either of the unilamellar or multilamellar nanoparticles can be further modified to be saturated or unsaturated with other lipid components, such as cholesterol, free fatty acids, and phospholipids, and also can include endogenous or added proteins and carbohydrates, such as cellular surface antigen. In such cases, an excess amount of the other lipid components

can be added to the membrane wall which will shed until the concentration in the membrane wall reaches equilibrium, which can be dependent upon the nanoparticle environment. Membranes may also comprise other agents that may or may not increase an activity of the nanoparticle. In other examples, functional groups such as antibodies and aptamers can be added to the outer surface of the membrane to enhance site targeting, such as to cell surface epitopes found in cancer cells. The membrane of the nanoparticles can also comprise particles that can be biodegradable, cationic nanoparticles including, but not limited to, gold, silver, and synthetic nanoparticles.

[00027] Synthetic or artificial membrane: As used herein, the term "synthetic membrane" or "artificial membrane" refers to a man-made membrane that is produced from organic material, such as polymers and liquids, as well as inorganic materials. A wide variety of synthetic membranes are well known in the art.

[00028] Nanoparticle: The term "nanoparticle" as used herein refers to nanostructure, particles, vesicles, or fragments thereof having at least one dimension (*e.g.*, height, length, width, or diameter) of between about 1 nm and about 10 μm. For systemic use, an average diameter of about 50 nm to about 500 nm, or 100 nm to 250 nm may be preferred. The term "nanostructure" includes, but is not necessarily limited to, particles and engineered features. The particles and engineered features can have, for example, a regular or irregular shape. Such particles are also referred to as nanoparticles. The nanoparticles can be composed of organic materials or other materials, and can alternatively be implemented with porous particles. The layer of nanoparticles can be implemented with nanoparticles in a monolayer or with a layer having agglomerations of nanoparticles. In some embodiments, the nanoparticle comprises or consists of an inner core covered by an outer surface comprising the membrane as discussed herein. The invention contemplates any nanoparticles now known and later developed that can be coated with the membrane described herein.

[00029] Pharmaceutically active: The term "pharmaceutically active" as used herein refers to the beneficial biological activity of a substance on living matter and, in particular, on cells and tissues of the human body. A "pharmaceutically active agent" or "drug" is a substance that is pharmaceutically active and a "pharmaceutically active ingredient" (PAI) is the pharmaceutically active substance in a drug.

[00030] Pharmaceutically acceptable: The term "pharmaceutically acceptable" as used herein means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia, other generally recognized pharmacopoeia in addition to other formulations that are safe for use in animals, and more particularly in humans and/or non-human mammals.

[00031] Pharmaceutically acceptable salt: The term "pharmaceutically acceptable salt" as used herein refers to acid addition salts or base addition salts of the compounds, such as the multi-drug conjugates, in the present disclosure. A pharmaceutically acceptable salt is any salt which retains the activity of the parent nanoparticle or compound and does not impart any deleterious or undesirable effect on a subject to whom it is administered and in the context in which it is administered. Pharmaceutically acceptable salts may be derived from amino acids including, but not limited to, cysteine. Methods for producing compounds as salts are known to those of skill in the art (*see*, for example, Stahl et al., Handbook of Pharmaceutical Salts: Properties, Selection, and Use, Wiley-VCH; Verlag Helvetica Chimica Acta, Zurich, 2002; Berge et al., J Pharm. Sci. 66: 1 , 1977). In some embodiments, a "pharmaceutically acceptable salt" is intended to mean a salt of a free acid or base of a nanoparticle or compound represented herein that is non-toxic, biologically tolerable, or otherwise biologically suitable for administration to the subject. *See, generally*, Berge, et al., J. Pharm. Sci., 1977, 66, 1 -19. Preferred pharmaceutically acceptable salts are those that are pharmacologically effective and suitable for contact with the tissues of subjects without undue toxicity, irritation, or allergic response. A nanoparticle or compound described herein may possess a sufficiently acidic group, a sufficiently basic group, both types of functional groups, or more than one of each type, and accordingly react with a number of inorganic or organic bases, and inorganic and organic acids, to form a pharmaceutically acceptable salt.

[00032] Examples of pharmaceutically acceptable salts include sulfates, pyrosul fates, bisulfates, sulfites, bisulfites, phosphates, monohydrogen-phosphates, dihydrogenphosphates, metaphosphates, pyrophosphates, chlorides, bromides, iodides, acetates, propionates, decanoates, caprylates, acrylates, formates, isobutyrates, caproates, heptanoates, propiolates, oxalates, malonates, succinates, suberates, sebacates, fumarates, maleates, butyne- 1 ,4-dioates, hexyne- 1 ,6-dioates, benzoates, chlorobenzoates,

methylbenzoates, dinitrobenzoates, hydroxybenzoates, methoxybenzoates, phthalates, sulfonates, methylsulfonates, propylsulfonates, besylates, xylenesulfonates, naphthalene- 1 -sulfonates, naphthalene-2-sulfonates, phenylacetates, phenylpropionates, phenylbutyrates, citrates, lactates, [gamma]-hydroxybutyrates, glycolates, tartrates, and mandelates.

[00033] Pharmaceutically acceptable carrier: The term "pharmaceutically acceptable carrier" as used herein refers to an excipient, diluent, preservative, solubilizer, emulsifier, adjuvant, and/or vehicle with which a nanoparticle or compound, such as a multi-drug conjugate, is administered. Such carriers may be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents. Antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; and agents for the adjustment of tonicity such as sodium chloride or dextrose may also be a carrier. Methods for producing compositions in combination with carriers are known to those of skill in the art. In some embodiments, the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. *See, e.g.*, Remington, The Science and Practice of Pharmacy. 20"' ed., (Lippincott, Williams & Wilkins 2003). Except insofar as any conventional media or agent is incompatible with the active compound, such use in the compositions is contemplated.

[00034] Phospholipid: The term "phospholipid", as used herein, refers to any of numerous lipids containing a diglyceride, a phosphate group, and a simple organic molecule such as choline. Examples of phospholipids include, but are not limited to, Phosphatide acid (phosphatidate) (PA), Phosphatidylethanolamine (cephalin) (PE), Phosphatidylcholine (lecithin) (PC), Phosphatidylserine (PS), and Phosphoinositides which include, but are not limited to, Phosphatidylinositol (PI), Phosphatidylinositol phosphate (PIP), Phosphatidylinositol bisphosphate (PIP2) and Phosphatidylinositol triphosphate (P1P3). Additional examples of PC include DDPC, DLPC, DMPC, DPPC, DSPC, DOPC, POPC, DRPC, and DEPC as defined in the art.

[00035] Therapeutically Effective Amount: As used herein, the term "therapeutically effective amount" refers to those amounts that, when administered to a particular subject in view of the nature and severity of that subject's disease or condition, will have a desired therapeutic effect, *e.g.*, an amount which will cure, prevent, inhibit, or at least partially arrest or partially prevent a target disease or condition. More specific embodiments are included in the Pharmaceutical Preparations and Methods of Administration section below. In some embodiments, the term "therapeutically effective amount" or "effective amount" refers to an amount of a therapeutic agent that when administered alone or in combination with an additional therapeutic agent to a cell, tissue, or subject is effective to prevent or ameliorate the disease or condition such as a hemolytic disease or condition, or the progression of the disease or condition. A therapeutically effective dose further refers to that amount of the therapeutic agent sufficient to result in amelioration of symptoms, *e.g.*, treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

[00036] "Treating" or "treatment" or "alleviation" refers to therapeutic treatment wherein the object is to slow down (lessen) if not cure the targeted pathologic condition or disorder or prevent recurrence of the condition. A subject is successfully "treated" if, after receiving a therapeutic amount of a therapeutic agent, the subject shows observable and/or measurable reduction in or absence of one or more signs and symptoms of the particular disease. Reduction of the signs or symptoms of a disease may also be felt by the patient. A patient is also considered treated if the patient experiences stable disease. In some embodiments, treatment with a therapeutic agent is effective to result in the patients being disease-free 3 months after treatment, preferably 6 months, more preferably one year, even more preferably 2 or more years post treatment. These parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician of appropriate skill in the art.

[00037] As used herein, "preventative" treatment is meant to indicate a postponement of development of a disease, a symptom of a disease, or medical condition, suppressing symptoms that may appear, or reducing the risk of developing or recurrence of a disease or symptom. "Curative" treatment includes reducing the severity of or suppressing the worsening of an existing disease, symptom, or condition.

[00038] The term "combination" refers to either a fixed combination in one dosage unit form, or a kit of parts for the combined administration where a nanoparticle or compound and a combination partner (*e.g.*, another drug as explained below, also referred to as "therapeutic agent" or "co-agent") may be administered independently at the same time or separately within time intervals, especially where these time intervals allow the combination partners to show a cooperative, *e.g.*, synergistic, effect. The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected combination partner to a single subject in need thereof (*e.g.*, a patient), and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time. The term "pharmaceutical combination" as used herein means a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients. The term "fixed combination" means that the active ingredients, *e.g.*, a nanoparticle or compound and a combination partner, are both administered to a patient simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that the active ingredients, *e.g.*, a nanoparticle or compound and a combination partner, are both administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the two moieties or compounds in the body of the patient. The latter also applies to cocktail therapy, *e.g.*, the administration of three or more active ingredients.

[00039] It is understood that aspects and embodiments of the invention described herein include "consisting" and/or "consisting essentially of" aspects and embodiments.

[00040] Throughout this disclosure, various aspects of this invention are presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the

scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[00041] As used herein, a subject in need refers to an animal, a non-human mammal or a human. As used herein, "animals" include a pet, a farm animal, an economic animal, a sport animal and an experimental animal, such as a cat, a dog, a horse, a cow, an ox, a pig, a donkey, a sheep, a lamb, a goat, a mouse, a rabbit, a chicken, a duck, a goose, a primate, including a monkey and a chimpanzee.

[00042] Other objects, advantages and features of the present invention will become apparent from the following specification taken in conjunction with the accompanying drawings.

B. Methods for preventing and/or treating infection by a platelet-targeting microbe in a subject

[00043] In one aspect, the present invention provides for a method for preventing and/or treating infection by a platelet-targeting microbe in a subject, which method comprises administering, to a subject in need, or to cells of said subject, an effective amount of a nanoparticle comprising a) an inner core comprising a non-cellular material, b) an outer surface comprising a cellular membrane derived from a platelet; and optionally c) an agent for preventing said infection, treating said infection, diagnosing said infection, prognosing said infection and/or monitoring prevention or treatment of said infection. In some embodiments, the infection is not an infection disclosed in U.S. Application Serial No. 13/827,906, filed March 14, 2013, International Application No. PCT/US2012/039411, filed May 24, 2012 and published as WO 2013/052167 A2 and U.S. provisional application Serial No. 61/492,626, filed June 2, 2011.

[00044] The present methods can be used to prevent and/or treat infection by a platelet-targeting microbe in any suitable subject. In some embodiments, the subject is a mammal.

In some embodiments, the mammal is a human. In other embodiments, the mammal is a non-human mammal, including a pet, a farm animal, an economic animal, a sport animal and an experimental animal, such as a cat, a dog, a horse, a cow, an ox, a pig, a donkey, a sheep, a lamb, a goat, a mouse, a rabbit, a primate, including a monkey and a chimpanzee.

[00045] In some embodiments, the present methods can be used for preventing infection by a platelet-targeting microbe in a subject. In other embodiments, the present methods can be used for treating infection by a platelet-targeting microbe in a subject. The present methods can be used to prevent and/or treat infection by a platelet-targeting microbe in a subject to any suitable degree. For example, present methods can be used to decrease or neutralize the effect of infection by a platelet-targeting microbe in a subject by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more compared to a comparable untreated subject or to the same subject at an untreated stage.

[00046] The nanoparticle used in the present methods can comprise any suitable inner core. For example, the inner core of the nanoparticle can comprise a polymeric particle core, a silica particle core, or a metal, *e.g.*, gold, particle core. Any suitable polymeric particle core can be used. In some embodiments, the polymeric particle core can comprise an optical shift property. In other embodiments, the polymeric particle core can comprise a metal, *e.g.*, gold, iron oxide or a quantum dot. In still other embodiments, the inner core of the nanoparticle can comprise a biocompatible or a synthetic material, such as poly(lactic-c -glycolic acid) (PLGA), polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL), polylysine, and polyglutamic acid. In yet other embodiments, the inner core of the nanoparticle supports the outer surface.

[00047] The nanoparticle used in the present methods can comprise any suitable cellular membrane derived from a platelet. For example, the nanoparticle used in the present methods can comprise a plasma membrane derived from a platelet. In another example, the nanoparticle used in the present methods can comprise an intracellular membrane derived from a platelet.

[00048] The therapeutic agent, the prophylactic agent, the diagnostic or marker agent, the prognostic agent, the isolation agent, the monitoring agent, or a combination thereof,

can be comprised in any suitable location in the composition used in the present methods. For example, the therapeutic agent, the prophylactic agent, the diagnostic or marker agent, the prognostic agent, the isolation agent, the monitoring agent, or a combination thereof, can be comprised in the nanoparticle. In some embodiments, the therapeutic agent, the prophylactic agent, the diagnostic or marker agent, the prognostic agent, the isolation agent, the monitoring agent, or a combination thereof, can be comprised in a releasable cargo in the nanoparticle.

[00049] The nanoparticle can comprise a releasable cargo at any suitable location. For example, the releasable cargo can be located within or on the inner core, between the inner core and the outer surface, or within or on the outer surface. The release of the releasable cargo can be triggered by any suitable mechanisms. For example, the release of the releasable cargo can be triggered by a contact between the nanoparticle and the subject or cells of the subject, or by a change of a physical parameter surrounding the nanoparticle. The nanoparticle can comprise any suitable type of a releasable cargo. For example, the releasable cargo can be a metallic particle, a polymeric particle, a dendrimer particle, or an inorganic particle.

[00050] In some embodiments, the therapeutic agent, the prophylactic agent, the diagnostic or marker agent, the prognostic agent, the isolation agent, the monitoring agent, or a combination thereof, can be comprised in the composition used in the present methods but outside the nanoparticle. In other embodiments, the therapeutic agent, the prophylactic agent, the diagnostic or marker agent, the prognostic agent, the isolation agent, the monitoring agent, or a combination thereof, can be administered to the subject separately from the composition used in the present methods. The composition used in the present methods and the additional substance can be administered to the subject simultaneously or sequentially.

[00051] In some embodiments, the therapeutic agent is used for treating infection by a platelet-targeting microbe in a subject. In other embodiments, the prophylactic agent is used for preventing infection by a platelet-targeting microbe in a subject. In still other embodiments, the diagnostic or marker agent is used for diagnosing infection by a platelet-targeting microbe in a subject. In yet other embodiments, the prognosing or marker agent is used for prognostic infection by a platelet-targeting microbe in a subject. In yet other

embodiments, the monitoring agent is used for monitoring prevention or treatment of infection by a platelet-targeting microbe in a subject. In yet other embodiments, the isolation agent is used for facilitating isolation and removal of a cell, tissue or organ associated with the infection from the subject. The isolation agent can comprise any suitable material. In some embodiments, the isolation agent comprises a magnetic material, *e.g.*, iron oxide.

[00052] The nanoparticle used in the present methods can have any suitable size. For example, the nanoparticle can have a diameter from about 10 nm to about 10 μm. In certain embodiments, the diameter of the nanoparticle is about 10 nm, 20 nm, 30 nm, 40 nm, 50 nm, 60 nm, 70nm, 80 nm, 90 nm, 100 nm, 110 nm, 120 nm, 130 nm, 140 nm, 150 nm, 200 nm, 300 nm, 400 nm, 500 nm, 600 nm, 700 nm, 800 nm, 900 nm, 1 μm, 2 μm, 3 μm, 4 μm, 5 μm, 6 μm, 7 μm, 8 μm, 9 μm, and 10 μm, or any sub-range within about 10 nm to about 10 μm, *e.g.*, any range between any two of the above sizes.

[00053] The nanoparticle used in the present methods can have any suitable shape, including but not limited to, sphere, square, rectangle, triangle, circular disc, cube-like shape, cube, rectangular parallelepiped (cuboid), cone, cylinder, prism, pyramid, right-angled circular cylinder and other regular or irregular shape.

[00054] In some embodiments, the nanoparticle used in the present methods substantially lacks constituents of the platelet from which the cellular membrane is derived. For example, the nanoparticle can lack 10%, 20%, 30%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the constituents of the platelet from which the cellular membrane is derived.

[00055] In some embodiments, the nanoparticle used in the present methods substantially maintains the natural structural integrity or activity of the cellular membrane derived from the platelet or the constituents of the cellular membrane derived from the platelet. For example, the nanoparticle can retain 10%, 20%, 30%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the natural structural integrity or activity for targeting a damaged or leaky vasculature in a subject.

[00056] In some embodiments, the nanoparticle used in the present methods is biocompatible or biodegradable. For example, the inner core of the nanoparticle comprises PLGA and the outer surface of the nanoparticle comprises a plasma membrane derived from the platelet.

[00057] The nanoparticle used in the present methods can have any suitable half-life *in vivo*. For example, the nanoparticle can have a half-life in blood circulation *in vivo* for at least from about 30 minutes to about 10 hours, *e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 hours.

[00058] The outer surface of the nanoparticle used in the present methods can further comprise a synthetic membrane. In some embodiments, the nanoparticles used in the present methods comprise a mixture of nanoparticles that comprise an outer surface comprising a platelet cellular membrane and nanoparticles that comprise an outer surface comprising a synthetic membrane. The nanoparticles that comprise an outer surface comprising a synthetic membrane may or may not be capable of targeting the microbe and/or its toxin in a subject. In some embodiments, both the nanoparticles that comprise an outer surface comprising a platelet cellular membrane and nanoparticles that comprise an outer surface comprising a synthetic membrane are capable of targeting the microbe and/or its toxin in a subject. In other embodiments, the nanoparticles that comprise an outer surface comprising a platelet cellular membrane is capable of targeting the microbe and/or its toxin in a subject, but the nanoparticles that comprise an outer surface comprising a synthetic membrane is not capable of targeting the microbe and/or its toxin in a subject.

[00059] The composition used in the present methods can comprise the nanoparticles that comprise an outer surface comprising a platelet cellular membrane and nanoparticles that comprise an outer surface comprising a synthetic membrane in any suitable ratio. In some embodiments, the present composition can comprise at least about 1% (w/w), 2% (w/w), 3% (w/w), 4% (w/w), 5% (w/w), 6% (w/w), 7% (w/w), 8% (w/w), 9% (w/w), 10% (w/w), 20% (w/w), 30% (w/w), 40% (w/w), 50% (w/w), 60% (w/w), 70% (w/w), 80% (w/w), 90% (w/w), 91% (w/w), 92% (w/w), 93% (w/w), 94% (w/w), 95% (w/w), 96% (w/w), 97% (w/w), 98% (w/w), 99% (w/w), or more of the nanoparticles that comprise an outer surface comprising a platelet cellular membrane. In other embodiments, the present composition can comprise at least about 1% (w/w), 2% (w/w), 3% (w/w), 4% (w/w), 5%

(w/w), 6% (w/w), 7% (w/w), 8% (w/w), 9% (w/w), 10% (w/w), 20% (w/w), 30% (w/w), 40% (w/w), 50% (w/w), 60% (w/w), 70% (w/w), 80% (w/w), 90% (w/w), 91% (w/w), 92% (w/w), 93% (w/w), 94% (w/w), 95% (w/w), 96% (w/w), 97% (w/w), 98% (w/w), 99% (w/w), or more of the nanoparticles that comprise an outer surface comprising a synthetic membrane. For example, the composition used in the present methods can comprise about 1-10% (w/w) of the nanoparticles that comprise an outer surface comprising a platelet cellular membrane and about 90-99% (w/w) of the nanoparticles that comprise an outer surface comprising a synthetic membrane, about 11-25% (w/w) of the nanoparticles that comprise an outer surface comprising a platelet cellular membrane and about 75-89% (w/w) of the nanoparticles that comprise an outer surface comprising a synthetic membrane, about 50% (w/w) of the nanoparticles that comprise an outer surface comprising a platelet cellular membrane and about 50% (w/w) of the nanoparticles that comprise an outer surface comprising a synthetic membrane, about 51-75% (w/w) of the nanoparticles that comprise an outer surface comprising a platelet cellular membrane and about 49-25% (w/w) of the nanoparticles that comprise an outer surface comprising a synthetic membrane, or about 90-100% (w/w) of the nanoparticles that comprise an outer surface comprising a platelet cellular membrane and about 0-10% (w/w) of the nanoparticles that comprise an outer surface comprising a synthetic membrane.

[00060] The outer surface of the nanoparticle can comprise a hybrid membrane comprising a cellular membrane derived from a platelet and a synthetic membrane. In some embodiments, the outer surface of the nanoparticle can comprise a hybrid membrane comprising at least about 5% (w/w), 6% (w/w), 7% (w/w), 8% (w/w), 9% (w/w), 10% (w/w), 20% (w/w), 30% (w/w), 40% (w/w), 50% (w/w), 60% (w/w), 70% (w/w), 80% (w/w), 90% (w/w), 91% (w/w), 92% (w/w), 93% (w/w), 94% (w/w), 95% (w/w), 96% (w/w), 97% (w/w), 98% (w/w), 99% (w/w) of a cellular membrane derived from a platelet. In other embodiments, the outer surface of the nanoparticle can comprise a hybrid membrane comprising at least about 1% (w/w), 2% (w/w), 3% (w/w), 4% (w/w), 5% (w/w), 6% (w/w), 7% (w/w), 8% (w/w), 9% (w/w), 10% (w/w), 20% (w/w), 30% (w/w), 40% (w/w), 50% (w/w), 60% (w/w), 70% (w/w), 80% (w/w), 90% (w/w), 91% (w/w), 92% (w/w), 93% (w/w), 94% (w/w), 95% (w/w) of a synthetic membrane. For example, the outer surface of the nanoparticle can comprise a hybrid membrane comprising about 5-10% (w/w) of a cellular membrane derived from a platelet and about 90-95% (w/w) of a

synthetic membrane, about 11-25% (w/w) of a cellular membrane derived from a platelet and about 75-89% (w/w) of a synthetic membrane, about 50% (w/w) of a cellular membrane derived from a platelet and about 50% (w/w) of a synthetic membrane, about 51-75% (w/w) of a cellular membrane derived from a platelet and about 49-25% (w/w) of a synthetic membrane, or about 90-99% (w/w) of a cellular membrane derived from a platelet and about 1-10% (w/w) of a synthetic membrane.

[00061] In some embodiments, the nanoparticle used in the present methods substantially lacks immunogenicity to the subject, *e.g.*, a mammal. For example, the cellular membrane can be derived from a platelet from the same species of the subject. In another example, the subject is a human and the cellular membrane is derived from a human platelet. In some embodiments, the cellular membrane can be derived from a platelet of the mammal to be treated. For example, the cellular membrane can be derived from a platelet of the human to be treated.

[00062] The present methods can be used for preventing and/or treating any suitable infection by a platelet-targeting microbe, effect of the microbial toxin and/or subject's response to the microbe or the microbial toxin in a subject. For example, the present methods can be used to reduce the microbial count or eliminate the microbe in a subject, to decrease or neutralize the effect of a toxin produced by the microbe in a subject, and/or to decrease or neutralize a subject's response to the microbe or the microbial toxin in the subject.

[00063] In some embodiments, the present methods can be used to reduce the microbial count or eliminate the microbe in a subject. In other embodiments, the present methods can be used to decrease or neutralize the effect of a toxin produced by the microbe in a subject. In still other embodiments, the present methods can be used to decrease or neutralize a subject's response to the microbe or the microbial toxin in the subject. The present methods can be used to decrease or neutralize a subject's response to the microbe or the microbial toxin that has harmful effect on the subject, *e.g.*, contributing to tissue damage of the subject, supporting microbial dissemination or supporting microbial survival in the subject. For example, the present methods can be used to decrease or neutralize a subject's response that results in thrombocytopenia, thrombosis, or enhancement of biofilm formation in the subject. The thrombocytopenia can be caused by

various mechanisms. For example, the thrombocytopenia can be caused by induction of activation and phagocytosis, *e.g.*, survival of platelets being shortened after activation by contact with pathogens; for example, in adenovirus infection, by HIV Tat protein, fungal pathogens (*Aspergillus*, *Candida* and *Mucormycetes*) and secreted compounds of *Aspergillus*; or STEC enhancing platelet phagocytosis by macrophages by inducing down modulation of platelet CD47. In another example, the thrombocytopenia can be caused by induction of apoptosis and cell lysis, *e.g.*, *Staphylococcus aureus* and *Escherichia coli*, as well as their secreted toxins, triggering degradation of the anti-apoptotic protein Bcl-x_L in platelets; Bacterial cell wall peptidoglycan stimulating platelet apoptosis; the toxins streptolysin O of *Streptococcus pyogenes* and pneumolysin of *Streptococcus pneumoniae* forming pores in the platelet membrane; Dengue virus infection inducing platelet apoptosis. In still another example, the thrombocytopenia can be caused by induction of antiplatelet autoimmune antibodies via molecular mimicry, *e.g.*, antibodies against microbial antigens of HIV, HCV, Dengue virus and *Helicobacter pylori* cross-reacting with platelet glycoproteins. In yet another example, the thrombocytopenia can be caused by affecting thrombopoiesis in the bone marrow, *e.g.*, HIV proteins interacting with the cell surface of platelet progenitor cells, thus inducing functional defects; mimicry of viral proteins inducing autoimmune antibodies that inhibit megakaryocyte differentiation; megakaryocytes infected by HIV, HCV, CMV and HHV-6; HIV modifying the cytokine pattern in the bone marrow that is necessary for thrombopoiesis; production of thrombopoietin, a growth factor for megakaryocyte differentiation, being impaired in HCV-induced liver disease. In yet another example, the thrombocytopenia can be caused by sequestration of platelets in the enlarged spleen, *e.g.*, HCV-induced portal hypertension resulting in platelet sequestration in the spleen.

[00064] The present methods can be used for preventing and/or treating any suitable infection by a platelet-targeting microbe or its toxin that binds to a platelet in the subject in any suitable way. In some embodiments, the present methods can be used for preventing and/or treating any suitable infection by a platelet-targeting microbe or its toxin that binds to a platelet in the subject directly. In other embodiments, the present methods can be used for preventing and/or treating any suitable infection by a platelet-targeting microbe or its toxin that binds to a platelet in the subject via a bridging moiety, *e.g.*, an antibody against the microbe or the microbial toxin.

[00065] The present methods can be used for preventing and/or treating any suitable infection by a platelet-targeting microbe or its toxin that binds to any suitable receptor on a platelet in the subject. For example, the present methods can be used for preventing and/or treating any suitable infection by a platelet-targeting microbe or its toxin that binds to GP1ba receptor, *e.g.*, direct binding of bacterial surface proteins or via vWF as bridging molecules; GPIa–IIa ($\alpha_2\beta_1$ integrin), *e.g.*, binding of rotavirus VP4 surface protein; GPIIb–IIIa ($\alpha_{2b}\beta_3$ integrin), *e.g.*, direct binding of bacterial surface proteins or via fibronectin and fibrinogen as bridging molecules; binding to adenovirus and hantavirus; GPVI, *e.g.*, binding of HCV; a toll-like receptor (TLR), *e.g.*, direct binding of bacterial LPS; FcγRIIa (CD32), *e.g.*, binding of IgG in immune complexes with all pathogens; a complement receptor, *e.g.*, binding of complement factors bound on pathogen surface; direct binding of EBV by CR2; binding of anaphylatoxins by C3aR and C5aR; a thrombin receptor, *e.g.*, bacteria-induced TF catalyzes thrombin generation that activates platelets; a cytokine/chemokine receptor, *e.g.*, sensing of inflammatory cytokines and chemokines; binding to HIV; a *N* -formyl peptide receptor, *e.g.*, binding of bacteria-derived formyl peptides with subsequent gradient-driven chemotaxis; a C-type lectin, *e.g.*, binding of HIV to DC-SIGN and CLEC-2; or a coxsackievirus and adenovirus receptor (CAR), *e.g.*, binding of adenovirus.

[00066] The present methods can be used for preventing and/or treating infection by any suitable platelet-targeting microbe in a subject. The platelet-targeting microbe can be a bacterium, a virus, a fungus and/or a parasite.

[00067] In some embodiments, the microbe can be a bacterium, *e.g.*, a Gram-positive bacterium or a Gram-negative bacterium. The present methods can be used for preventing and/or treating infection by any suitable platelet-targeting bacterium in a subject. In some embodiments, the bacterium can be in a genus of *Staphylococcus*, *Escherichia*, *Streptococcus* or *Helicobacter*. For example, the bacterium can be in the genus of *Staphylococcus*. The *Staphylococcus* bacterium can be in *S. aureus* group (*e.g.*, *S. aureus* or *S. simiae*), *S. auricularis* group (*e.g.*, *S. auricularis*), *S. carnosus* group (*e.g.*, *S. carnosus*, *S. condimenti*, *S. massiliensis*, *S. piscifermentans*, or *S. simulans*), *S. epidermidis* group (*e.g.*, *S. capitis*, *S. caprae*, *S. epidermidis*, or *S. saccharolyticus*), *S. haemolyticus* group (*e.g.*, *S. devriesei*, *S. haemolyticus*, or *S. hominis*), *S. hyicus-intermedius* group (*e.g.*,

S. chromogenes, *S. felis*, *S. delphini*, *S. hyicus*, *S. intermedius*, *S. lutrae*, *S. microti*, *S. muscae*, *S. pseudintermedius*, *S. rostri*, or *S. schleiferi*), *S. lugdunensis* group (*e.g.*, *S. lugdunensis*), *S. saprophyticus* group (*e.g.*, *S. arlettae*, *S. cohnii*, *S. equorum*, *S. gallinarum*, *S. kloosii*, *S. leei*, *S. nepalensis*, *S. saprophyticus*, *S. succinus*, or *S. xylosus*), *S. sciuri* group (*e.g.*, *S. fleurettii*, *S. lentus*, *S. sciuri*, *S. stepanovicii*, or *S. vitulinus*), *S. simulans* group (*e.g.*, *S. simulans*) or *S. warneri* group (*e.g.*, *S. pasteuri*, or *S. warneri*). In another example, the bacterium can be in the genus of *Escherichia*, *e.g.*, *Escherichia coli*. In still another example, the bacterium can be in the genus of *Streptococcus*. The *Streptococcus* bacterium can be *Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus equisimilis, Streptococcus bovis, Streptococcus anginosus, Streptococcus sanguinis, Streptococcus suis, Streptococcus mitis, Streptococcus mutans,* or *Streptococcus pneumoniae.* In yet another example, the bacterium can be in the genus of *Helicobacter*. The *Helicobacter* bacterium can be *Helicobacter pylori* or a non-*pylori Helicobacter* species. The non-*pylori Helicobacter* species can be *H. suis*, *H. baculiformis*, *H. equorum*, *H. hepaticus, H. mustelae*, *H. bilis, H. felis, H. bizzozeronii*, *H. salomonis, H. ganmani*, *H. pullorum, H. anseris*, *H. brantae, H. cinaedi* or *H. canis*.

[00068] In some embodiments, the nanoparticle can comprise an agent for preventing the bacterial infection, treating the bacterial infection, diagnosing the bacterial infection, prognosing the bacterial infection and/or monitoring prevention or treatment of the bacterial infection. Any suitable agent can be used. In some embodiments, the agent is an antibiotic. Any suitable antibiotic can be used. For example, the antibiotic can have a bactericidal activity or bacteriostatic activity. In another example, the antibiotic can be a narrow-spectrum or a broad-spectrum antibiotic. In still another example, the antibiotic can target the bacterial cell wall (*e.g.*, penicillins and cephalosporins), can target the cell membrane (*e.g.*, polymyxins), can interfere with essential bacterial enzymes (*e.g.*, rifamycins, lipiarmycins, quinolones and sulfonamides), targets protein synthesis (*e.g.*, macrolides, lincosamides and tetracyclines), or can be a cyclic lipopeptide (such as daptomycin), a glycylcycline (such as tigecycline), an oxazolidinone (such as linezolid), or a lipiarmycin (such as fidaxomicin). In other embodiments, the nanoparticle can comprise an agent for decreasing or neutralizing the effect of a bacterial toxin, *e.g.*, a RBC coated nanoparticle. *See e.g.*, U.S. Application Serial No. 13/827,906, filed March 14, 2013, International Application No. PCT/US2012/039411, filed May 24, 2012 and published as

WO 2013/052167 A2 and U.S. provisional application Serial No. 61/492,626, filed June 2, 2011.

[00069] In some embodiments, the microbe can be a virus. The present methods can be used for preventing and/or treating infection by any suitable platelet-targeting virus in a subject. In some embodiments, the virus can be a dsDNA virus (*e.g.*, an adenoviruse, a herpesvirus, a poxvirus), a ssDNA virus (+ strand or "sense") DNA (*e.g.*, a parvovirus), a dsRNA virus (*e.g.*, a reovirus), (+)ssRNA virus (+ strand or sense) RNA (*e.g.*, a picornavirus, a togavirus) or a (-)ssRNA virus (– strand or antisense) RNA (*e.g.*, an orthomyxovirus, a rhabdovirus), a ssRNA-RT virus (+ strand or sense) RNA with DNA intermediate in life-cycle (*e.g.*, a retrovirus) or a dsDNA-RT virus (*e.g.*, a hepadnavirus). In other embodiments, the virus can be an adenovirus (*e.g.*, adenovirus serotypes 1, 2, 4, 5, 6, 7, 8, 14, 19, 36, 37, 40 and 41), a coxsackievirus, a cytomegalovirus (CMV) (*e.g.*, human CMV or human herpesvirus-5 (HHV-5)), a Dengue virus, a hantavirus (*e.g.*, a strain that causes hantavirus hemorrhagic fever with renal syndrome (HFRS) or Hantavirus pulmonary syndrome (HPS)), a hepatitis C virus (HCV), a hepatitis B virus (HBV), a herpesviridae (*e.g.*, Epstein–Barr virus (EBV), also called human herpesvirus 4 (HHV-4)), a lentivirus (*e.g.*, the human immunodeficiency virus (HIV)), a muromegalovirus (*e.g.*, human herpesvirus 6 (HHV-6)), a Roseolovirus (*e.g.*, HHV-6 and human herpesvirus 7 (HHV-7)), or a rotavirus (A, B, C, D, and E rotavirus A).

[00070] In some embodiments, the nanoparticle can comprise an agent, *e.g.*, an anti-viral agent, for preventing the viral infection, treating the viral infection, diagnosing the viral infection, prognosing the viral infection and/or monitoring prevention or treatment of the viral infection. Any suitable anti-viral agent can be used. For example, the anti-viral agent can interfere with the ability of a virus to infiltrate a target cell (*e.g.*, an entry inhibitor and an uncoating inhibitor), can target the processes that synthesize virus components after a virus invades a cell (*e.g.*, an inhibitor or viral reverse transcription, integrase, transcription, translation, protein processing and targeting, or a protease inhibitor), can target viral assembly or can target viral release phase.

[00071] In some embodiments, the microbe can be a fungus. The present methods can be used for preventing and/or treating infection by any suitable platelet-targeting fungus in a subject. For example, the fungus can be a genus of *Candida* (*e.g.*, *Candida albicans*),

Aspergillus (*e.g.*, *Aspergillus fumigatus* and *Aspergillus flavus*), *Cryptococcus* (*e.g.*, *Cryptococcus neoformans* and *Cryptococcus gattii*), *Histoplasma* (*e.g.*, *Histoplasma capsulatum*), *Mucormycetes* (*e.g.*, *Rhizopus oryzae* (or *Rhizopus microsporus*), *Mucor* species, *Lichtheimia corymbifera*, and *Rhizomucor pusillus*), *Pneumocystis* (*e.g.*, *Pneumocystis jirovecii*), or *Stachybotrys* (*e.g.*, *Stachybotrys chartarum*). In some embodiments, the nanoparticle can comprise an agent, *e.g.*, an anti-fungal agent, for preventing the fungal infection, treating the fungal infection, diagnosing the fungal infection, prognosing the fungal infection and/or monitoring prevention or treatment of the fungal infection. Any suitable anti- fungal agent can be used. For example, the anti-fungal agent can be a polyene antifungal agent, an imidazole antifungal agent, a triazole antifungal agent, a thiazole antifungal agent, an allylamine or an echinocandin.

[00072] In some embodiments, the microbe can be a parasite. The present methods can be used for preventing and/or treating infection by any suitable platelet-targeting parasite in a subject. For example, the parasite can be in a genus of *Plasmodium* (*e.g.*, *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesii*), *Schistosoma* (*Schistosoma guineensis*, *Schistosoma intercalatum*, *Schistosoma haematobium*, *Schistosoma japonicum*, *Schistosoma malayensis*, *Schistosoma mansoni* and *Schistosoma mekongi*) or *Toxoplasma gondii*. In some embodiments, the nanoparticle can comprise an agent, *e.g.*, an anti-parasitic agent, for preventing the parasitic infection, treating the parasitic infection, diagnosing the parasitic infection, prognosing the parasitic infection and/or monitoring prevention or treatment of the parasitic infection. Any suitable anti-parasitic agent can be used. For example, the anti-parasitic agent can be an anti-worm agent, an anti-malaria agent or an anti-*Toxoplasma gondii* agent. Any suitable anti-malaria agent can be used. For example, the anti-malaria agent can be used for treating malaria in a subject with suspected or confirmed infection, for preventing infection in a subject, or for routine intermittent treatment of certain groups of subjects in an endemic region. In another example, the anti-malaria agent can be a quinine and related agent, chloroquine, amodiaquine, pyrimethamine, proguanil, a sulfonamide, mefloquine, atovaquone, primaquine, artemisinin and derivative, halofantrine, doxycycline, or clindamycin.

[00073] The present methods can further comprise administering another active ingredient to the subject. The other active ingredient can be used for preventing and/or

treating infection by a platelet-targeting microbe in a subject. Any suitable active ingredient can be used. For example, the other active ingredient can comprise a red blood cell membrane coated nanoparticle comprising an inner core comprising a non-cellular material and an outer surface comprising a cellular membrane derived from a red blood cell. The red blood cell membrane coated nanoparticle can be used to decrease or neutralize the effect of a RBC-targeting toxin produced by the microbe in a subject. The red blood cell membrane coated nanoparticle can further comprise active ingredient for preventing and/or treating infection by a microbe in a subject. The red blood cell membrane coated nanoparticle can also comprise active ingredient for preventing and/or treating infection by a RBC-targeting microbe in a subject.

[00074] In one example, the infection to be prevented and/or treated is infection by a platelet-targeting microbe that also produces a RBC-targeting toxin. One such exemplary microbe is *S. aureus*, *e.g.*, methicillin-resistant *Staphylococcus aureus* (MRSA), which produces RBC-targeting alpha-toxin (also known as hemolysin). The present methods can use a combination of a platelet coated nanoparticle, optionally containing an antibiotic, to reduce the microbial count or eliminate the microbe in a subject, and a RBC coated nanoparticle to decrease or neutralize the effect of a toxin produced by the microbe in a subject.

[00075] In some embodiments, the present methods can further comprise administering a pharmaceutically acceptable carrier or excipient to the subject.

[00076] The composition used in the present methods can be administered using any suitable delivery mechanisms or techniques. In some embodiments, the composition can be administered alone. In other embodiments, the composition can be administered with a pharmaceutically acceptable carrier or excipient. In still other embodiments, the composition can be administered via a medicament delivery system or a medical device. Any suitable medicament delivery system or medical device can be used. For example, the medicament delivery system or the medical device can be an implant, *e.g.*, an implant placed during or after bone surgery, a catheter, or a sustained-release drug delivery system.

[00077] In some embodiments, the present methods can further comprise assessing efficacy of the nanoparticle and/or the other active ingredient in preventing and/or treating infection by a platelet-targeting microbe in a subject. The efficacy of the nanoparticle and/or the other active ingredient in preventing and/or treating infection by a platelet-targeting microbe can be assessed by any suitable methods, *e.g.*, *in vitro* and/or *in vivo* tests. For example, the present methods can be used for preventing and/or treating any suitable infection by a platelet-targeting microbe, *e.g.*, bacterium, virus, fungus and/or parasite, and the efficacy of the nanoparticle and/or the other active ingredient can be assessed by any suitable measurement of microbial and/or its toxin count in the treated subject relative to the untreated status or subject.

[00078] The composition used in the present methods can be administered to the subject via any suitable route of administration. In some embodiments, the nanoparticle used in the present methods, alone or in combination with other active ingredient(s), can be administered via oral, parenteral, rectal, nasal, topical, or ocular routes, or by inhalation. Exemplary parenteral administration can be via intravenous, intramuscular, intraperitoneal, intranasal, or subcutaneous route.

[00079] In another aspect, the present invention is directed to use of an effective amount of a nanoparticle for the manufacture of a medicament for preventing and/or treating infection by a platelet-targeting microbe in a subject, wherein said nanoparticle comprises: a) an inner core comprising a non-cellular material, b) an outer surface comprising a cellular membrane derived from a platelet; and optionally c) an agent for preventing said infection, treating said infection, diagnosing said infection, prognosing said infection and/or monitoring prevention or treatment of said infection.

C. Combinations for preventing and/or treating infection by a platelet-targeting microbe in a subject

[00080] In still another aspect, the present invention is directed to a combination for preventing and/or treating infection by a platelet-targeting microbe in a subject, which combination comprises an effective amount of a nanoparticle and an effective amount of a second prophylactic or therapeutic agent for preventing and/or treating infection by a platelet-targeting microbe in a subject, wherein said nanoparticle comprises: a) an inner

core comprising a non-cellular material, b) an outer surface comprising a cellular membrane derived from a platelet; and optionally c) an agent for preventing said infection, treating said infection, diagnosing said infection, prognosing said infection and/or monitoring prevention or treatment of said infection. In some embodiments, the infection is not infection disclosed in U.S. Application Serial No. 13/827,906, filed March 14, 2013, International Application No. PCT/US2012/039411, filed May 24, 2012 and published as WO 2013/052167 A2 and U.S. provisional application Serial No. 61/492,626, filed June 2, 2011.

[00081] The present combination can be made, stored and/or used in any suitable formulation. In some embodiments, the present invention provides for a pharmaceutical composition comprising the above combination admixed with at least one pharmaceutically acceptable carrier or excipient. In other embodiments, the present invention provides for a method for preventing and/or treating infection by a platelet-targeting microbe in a subject, which method comprises administering, to a subject in need, or to cells of said subject, an effective amount of the above combination or pharmaceutical composition.

[00082] The above combination or pharmaceutical composition can be used to prevent and/or treat infection by a platelet-targeting microbe in any suitable subject. In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In other embodiments, the mammal is a non-human mammal, including a pet, a farm animal, an economic animal, a sport animal and an experimental animal, such as a cat, a dog, a horse, a cow, an ox, a pig, a donkey, a sheep, a lamb, a goat, a mouse, a rabbit, a primate, including a monkey and a chimpanzee.

[00083] In some embodiments, the above combination or pharmaceutical composition can be used for preventing infection by a platelet-targeting microbe in a subject. In other embodiments, the above combination or pharmaceutical composition can be used for treating infection by a platelet-targeting microbe in a subject. The above combination or pharmaceutical composition can be used to prevent and/or treat infection by a platelet-targeting microbe in a subject to any suitable degree. For example, the above combination or pharmaceutical composition can be used to decrease or neutralize the effect of infection by a platelet-targeting microbe in a subject by at least 10%, 20%, 30%, 40%, 50%, 60%,

70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more compared to a comparable untreated subject or to the same subject at an untreated stage.

[00084] The nanoparticle used in the above combination or pharmaceutical composition can comprise any suitable inner core. For example, the inner core of the nanoparticle can comprise a polymeric particle core, a silica particle core, or a metal, *e.g.*, gold, particle core. Any suitable polymeric particle core can be used. In some embodiments, the polymeric particle core can comprise an optical shift property. In other embodiments, the polymeric particle core can comprise a metal, *e.g.*, gold, iron oxide or a quantum dot. In still other embodiments, the inner core of the nanoparticle can comprise a biocompatible or a synthetic material, such as poly(lactic-c -glycolic acid) (PLGA), polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL), polylysine, and polyglutamic acid. In yet other embodiments, the inner core of the nanoparticle supports the outer surface.

[00085] The nanoparticle used in the above combination or pharmaceutical composition can comprise any suitable cellular membrane derived from a platelet. For example, the nanoparticle used in the above combination or pharmaceutical composition can comprise a plasma membrane derived from a platelet. In another example, the nanoparticle used in the above combination or pharmaceutical composition can comprise an intracellular membrane derived from a platelet.

[00086] The therapeutic agent, the prophylactic agent, the diagnostic or marker agent, the prognostic agent, the isolation agent, the monitoring agent, or a combination thereof, can be comprised in any suitable location in the above combination or pharmaceutical composition. For example, the therapeutic agent, the prophylactic agent, the diagnostic or marker agent, the prognostic agent, the isolation agent, the monitoring agent, or a combination thereof, can be comprised in the nanoparticle. In some embodiments, the therapeutic agent, the prophylactic agent, the diagnostic or marker agent, the prognostic agent, the isolation agent, the monitoring agent, or a combination thereof, can be comprised in a releasable cargo in the nanoparticle.

[00087] The nanoparticle can comprise a releasable cargo at any suitable location. For example, the releasable cargo can be located within or on the inner core, between the inner

core and the outer surface, or within or on the outer surface. The release of the releasable cargo can be triggered by any suitable mechanisms. For example, the release of the releasable cargo can be triggered by a contact between the nanoparticle and the subject or cells of the subject, or by a change of a physical parameter surrounding the nanoparticle. The nanoparticle can comprise any suitable type of a releasable cargo. For example, the releasable cargo can be a metallic particle, a polymeric particle, a dendrimer particle, or an inorganic particle.

[00088] In some embodiments, the therapeutic agent, the prophylactic agent, the diagnostic or marker agent, the prognostic agent, the isolation agent, the monitoring agent, or a combination thereof, can be comprised in the above combination or pharmaceutical composition but outside the nanoparticle.

[00089] In some embodiments, the therapeutic agent is used for treating infection by a platelet-targeting microbe in a subject. In other embodiments, the prophylactic agent is used for preventing infection by a platelet-targeting microbe in a subject. In still other embodiments, the diagnostic or marker agent is used for diagnosing infection by a platelet-targeting microbe in a subject. In yet other embodiments, the prognosing or marker agent is used for prognostic infection by a platelet-targeting microbe in a subject. In yet other embodiments, the monitoring agent is used for monitoring prevention or treatment of infection by a platelet-targeting microbe in a subject. In yet other embodiments, the isolation agent is used for facilitating isolation and removal of a cell, tissue or organ associated with the infection from the subject. The isolation agent can comprise any suitable material. In some embodiments, the isolation agent comprises a magnetic material, *e.g.*, iron oxide.

[00090] The nanoparticle used in the above combination or pharmaceutical composition can have any suitable size. For example, the nanoparticle can have a diameter from about 10 nm to about 10 μm. In certain embodiments, the diameter of the nanoparticle is about 10 nm, 20 nm, 30 nm, 40 nm, 50 nm, 60 nm, 70nm, 80 nm, 90 nm, 100 nm, 110 nm, 120 nm, 130 nm, 140 nm, 150 nm, 200 nm, 300 nm, 400 nm, 500 nm, 600 nm, 700 nm, 800 nm, 900 nm, 1 μm, 2 μm, 3 μm, 4 μm, 5 μm, 6 μm, 7 μm, 8 μm, 9 μm, and 10 μm, or any sub-range within about 10 nm to about 10 μm, *e.g.*, any range between any two of the above sizes.

[00091] The nanoparticle used in the above combination or pharmaceutical composition can have any suitable shape, including but not limited to, sphere, square, rectangle, triangle, circular disc, cube-like shape, cube, rectangular parallelepiped (cuboid), cone, cylinder, prism, pyramid, right-angled circular cylinder and other regular or irregular shape.

[00092] In some embodiments, the nanoparticle used in the above combination or pharmaceutical composition substantially lacks constituents of the platelet from which the cellular membrane is derived. For example, the nanoparticle can lack 10%, 20%, 30%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the constituents of the platelet from which the cellular membrane is derived.

[00093] In some embodiments, the nanoparticle used in the above combination or pharmaceutical composition substantially maintains the natural structural integrity or activity of the cellular membrane derived from the platelet or the constituents of the cellular membrane derived from the platelet. For example, the nanoparticle can retain 10%, 20%, 30%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the natural structural integrity or activity for targeting a platelet-targeting microbe in a subject.

[00094] In some embodiments, the nanoparticle used in the above combination or pharmaceutical composition is biocompatible or biodegradable. For example, the inner core of the nanoparticle comprises PLGA and the outer surface of the nanoparticle comprises a plasma membrane derived from the platelet.

[00095] The nanoparticle used in the above combination or pharmaceutical composition can have any suitable half-life *in vivo*. For example, the nanoparticle can have a half-life in blood circulation *in vivo* for at least from about 30 minutes to about 10 hours, *e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 hours.

[00096] The outer surface of the nanoparticle used in the above combination or pharmaceutical composition can further comprise a synthetic membrane. In some embodiments, the nanoparticles used in the above combination or pharmaceutical composition comprise a mixture of nanoparticles that comprise an outer surface

comprising a platelet cellular membrane and nanoparticles that comprise an outer surface comprising a synthetic membrane. The nanoparticles that comprise an outer surface comprising a synthetic membrane may or may not be capable of targeting the microbe and/or its toxin in a subject. In some embodiments, both the nanoparticles that comprise an outer surface comprising a platelet cellular membrane and nanoparticles that comprise an outer surface comprising a synthetic membrane are capable of targeting the microbe and/or its toxin in a subject. In other embodiments, the nanoparticles that comprise an outer surface comprising a platelet cellular membrane is capable of targeting the microbe and/or its toxin in a subject, but the nanoparticles that comprise an outer surface comprising a synthetic membrane is not capable of targeting the microbe and/or its toxin in a subject.

[00097] The composition used in the above combination or pharmaceutical composition can comprise the nanoparticles that comprise an outer surface comprising a platelet cellular membrane and nanoparticles that comprise an outer surface comprising a synthetic membrane in any suitable ratio. In some embodiments, the present composition can comprise at least about 1% (w/w), 2% (w/w), 3% (w/w), 4% (w/w), 5% (w/w), 6% (w/w), 7% (w/w), 8% (w/w), 9% (w/w), 10% (w/w), 20% (w/w), 30% (w/w), 40% (w/w), 50% (w/w), 60% (w/w), 70% (w/w), 80% (w/w), 90% (w/w), 91% (w/w), 92% (w/w), 93% (w/w), 94% (w/w), 95% (w/w), 96% (w/w), 97% (w/w), 98% (w/w), 99% (w/w), or more of the nanoparticles that comprise an outer surface comprising a platelet cellular membrane. In other embodiments, the present composition can comprise at least about 1% (w/w), 2% (w/w), 3% (w/w), 4% (w/w), 5% (w/w), 6% (w/w), 7% (w/w), 8% (w/w), 9% (w/w), 10% (w/w), 20% (w/w), 30% (w/w), 40% (w/w), 50% (w/w), 60% (w/w), 70% (w/w), 80% (w/w), 90% (w/w), 91% (w/w), 92% (w/w), 93% (w/w), 94% (w/w), 95% (w/w), 96% (w/w), 97% (w/w), 98% (w/w), 99% (w/w), or more of the nanoparticles that comprise an outer surface comprising a synthetic membrane. For example, the composition used in the above combination or pharmaceutical composition can comprise about 1-10% (w/w) of the nanoparticles that comprise an outer surface comprising a platelet cellular membrane and about 90-99% (w/w) of the nanoparticles that comprise an outer surface comprising a synthetic membrane, about 11-25% (w/w) of the nanoparticles that comprise an outer surface comprising a platelet cellular membrane and about 75-89% (w/w) of the nanoparticles that comprise an outer surface comprising a synthetic

membrane, about 50% (w/w) of the nanoparticles that comprise an outer surface comprising a platelet cellular membrane and about 50% (w/w) of the nanoparticles that comprise an outer surface comprising a synthetic membrane, about 51-75% (w/w) of the nanoparticles that comprise an outer surface comprising a platelet cellular membrane and about 49-25% (w/w) of the nanoparticles that comprise an outer surface comprising a synthetic membrane, or about 90-100% (w/w) of the nanoparticles that comprise an outer surface comprising a platelet cellular membrane and about 0-10% (w/w) of the nanoparticles that comprise an outer surface comprising a synthetic membrane.

[00098] The composition used in the above combination or pharmaceutical composition can comprise the nanoparticles that comprise an outer surface comprising a hybrid membrane comprising a cellular membrane derived from a platelet and a synthetic membrane. In some embodiments, the outer surface of the nanoparticle can comprise a hybrid membrane comprising at least about 5% (w/w), 6% (w/w), 7% (w/w), 8% (w/w), 9% (w/w), 10% (w/w), 20% (w/w), 30% (w/w), 40% (w/w), 50% (w/w), 60% (w/w), 70% (w/w), 80% (w/w), 90% (w/w), 91% (w/w), 92% (w/w), 93% (w/w), 94% (w/w), 95% (w/w), 96% (w/w), 97% (w/w), 98% (w/w), 99% (w/w) of a cellular membrane derived from a platelet. In other embodiments, the outer surface of the nanoparticle can comprise a hybrid membrane comprising at least about 1% (w/w), 2% (w/w), 3% (w/w), 4% (w/w), 5% (w/w), 6% (w/w), 7% (w/w), 8% (w/w), 9% (w/w), 10% (w/w), 20% (w/w), 30% (w/w), 40% (w/w), 50% (w/w), 60% (w/w), 70% (w/w), 80% (w/w), 90% (w/w), 91% (w/w), 92% (w/w), 93% (w/w), 94% (w/w), 95% (w/w) of a synthetic membrane. For example, the outer surface of the nanoparticle can comprise a hybrid membrane comprising about 5-10% (w/w) of a cellular membrane derived from a platelet and about 90-95% (w/w) of a synthetic membrane, about 11-25% (w/w) of a cellular membrane derived from a platelet and about 75-89% (w/w) of a synthetic membrane, about 50% (w/w) of a cellular membrane derived from a platelet and about 50% (w/w) of a synthetic membrane, about 51-75% (w/w) of a cellular membrane derived from a platelet and about 49-25% (w/w) of a synthetic membrane, or about 90-99% (w/w) of a cellular membrane derived from a platelet and about 1-10% (w/w) of a synthetic membrane.

[00099] In some embodiments, the nanoparticle used in the above combination or pharmaceutical composition substantially lacks immunogenicity to the subject, *e.g.*, a

mammal. For example, the cellular membrane can be derived from a platelet from the same species of the subject. In another example, the subject is a human and the cellular membrane is derived from a human platelet. In some embodiments, the cellular membrane can be derived from a platelet of the mammal to be treated. For example, the cellular membrane can be derived from a platelet of the human to be treated.

[000100] The above combination or pharmaceutical composition can be used for preventing and/or treating any suitable infection by a platelet-targeting microbe, effect of the microbial toxin and/or subject's response to the microbe or the microbial toxin in a subject. For example, the above combination or pharmaceutical composition can be used to reduce the microbial count or eliminate the microbe in a subject, to decrease or neutralize the effect of a toxin produced by the microbe in a subject, and/or to decrease or neutralize a subject's response to the microbe or the microbial toxin in the subject.

[000101] In some embodiments, the above combination or pharmaceutical composition can be used to reduce the microbial count or eliminate the microbe in a subject. In other embodiments, the above combination or pharmaceutical composition can be used to decrease or neutralize the effect of a toxin produced by the microbe in a subject. In still other embodiments, the above combination or pharmaceutical composition can be used to decrease or neutralize a subject's response to the microbe or the microbial toxin in the subject. The above combination or pharmaceutical composition can be used to decrease or neutralize a subject's response to the microbe or the microbial toxin that has harmful effect on the subject, *e.g.*, contributing to tissue damage of the subject, supporting microbial dissemination or supporting microbial survival in the subject. For example, the above combination or pharmaceutical composition can be used to decrease or neutralize a subject's response that results in thrombocytopenia, thrombosis, or enhancement of biofilm formation in the subject. The thrombocytopenia can be caused by various mechanisms. For example, the thrombocytopenia can be caused by induction of activation and phagocytosis, *e.g.*, survival of platelets being shortened after activation by contact with pathogens; for example, in adenovirus infection, by HIV Tat protein, fungal pathogens (*Aspergillus*, *Candida* and *Mucormycetes*) and secreted compounds of *Aspergillus*; or STEC enhancing platelet phagocytosis by macrophages by inducing down modulation of platelet CD47. In another example, the thrombocytopenia can be caused by

induction of apoptosis and cell lysis, *e.g.*, *Staphylococcus aureus* and *Escherichia coli*, as well as their secreted toxins, triggering degradation of the anti-apoptotic protein Bcl-x_L in platelets; Bacterial cell wall peptidoglycan stimulating platelet apoptosis; the toxins streptolysin O of *Streptococcus pyogenes* and pneumolysin of *Streptococcus pneumoniae* forming pores in the platelet membrane; Dengue virus infection inducing platelet apoptosis. In still another example, the thrombocytopenia can be caused by induction of antiplatelet autoimmune antibodies via molecular mimicry, *e.g.*, antibodies against microbial antigens of HIV, HCV, Dengue virus and *Helicobacter pylori* cross-reacting with platelet glycoproteins. In yet another example, the thrombocytopenia can be caused by affecting thrombopoiesis in the bone marrow, *e.g.*, HIV proteins interacting with the cell surface of platelet progenitor cells, thus inducing functional defects; mimicry of viral proteins inducing autoimmune antibodies that inhibit megakaryocyte differentiation; megakaryocytes infected by HIV, HCV, CMV and HHV-6; HIV modifying the cytokine pattern in the bone marrow that is necessary for thrombopoiesis; production of thrombopoietin, a growth factor for megakaryocyte differentiation, being impaired in HCV-induced liver disease. In yet another example, the thrombocytopenia can be caused by sequestration of platelets in the enlarged spleen, *e.g.*, HCV-induced portal hypertension resulting in platelet sequestration in the spleen.

[000102] The above combination or pharmaceutical composition can be used for preventing and/or treating any suitable infection by a platelet-targeting microbe or its toxin that binds to a platelet in the subject in any suitable way. In some embodiments, the above combination or pharmaceutical composition can be used for preventing and/or treating any suitable infection by a platelet-targeting microbe or its toxin that binds to a platelet in the subject directly. In other embodiments, the above combination or pharmaceutical composition can be used for preventing and/or treating any suitable infection by a platelet-targeting microbe or its toxin that binds to a platelet in the subject via a bridging moiety, *e.g.*, an antibody against the microbe or the microbial toxin.

[000103] The above combination or pharmaceutical composition can be used for preventing and/or treating any suitable infection by a platelet-targeting microbe or its toxin that binds to any suitable receptor on a platelet in the subject. For example, the above combination or pharmaceutical composition can be used for preventing and/or treating any

suitable infection by a platelet-targeting microbe or its toxin that binds to GP1ba receptor, *e.g.*, direct binding of bacterial surface proteins or via vWF as bridging molecules; GPIa–IIa ($\alpha_2\beta_1$ integrin), *e.g.*, binding of rotavirus VP4 surface protein; GPIIb–IIIa ($\alpha_{2b}\beta_3$ integrin), *e.g.*, direct binding of bacterial surface proteins or via fibronectin and fibrinogen as bridging molecules; binding to adenovirus and hantavirus; GPVI, *e.g.*, binding of HCV; a toll-like receptor (TLR), *e.g.*, direct binding of bacterial LPS; FcγRIIa (CD32), *e.g.*, binding of IgG in immune complexes with all pathogens; a complement receptor, *e.g.*, binding of complement factors bound on pathogen surface; direct binding of EBV by CR2; binding of anaphylatoxins by C3aR and C5aR; a thrombin receptor, *e.g.*, bacteria-induced TF catalyzes thrombin generation that activates platelets; a cytokine/chemokine receptor, *e.g.*, sensing of inflammatory cytokines and chemokines; binding to HIV; a *N* -formyl peptide receptor, *e.g.*, binding of bacteria-derived formyl peptides with subsequent gradient-driven chemotaxis; a C-type lectin, *e.g.*, binding of HIV to DC-SIGN and CLEC-2; or a coxsackievirus and adenovirus receptor (CAR), *e.g.*, binding of adenovirus.

[000104] The above combination or pharmaceutical composition can be used for preventing and/or treating infection by any suitable platelet-targeting microbe in a subject. The platelet-targeting microbe can be a bacterium, a virus, a fungus and/or a parasite.

[000105] In some embodiments, the microbe can be a bacterium, *e.g.*, a Gram-positive bacterium or a Gram-negative bacterium. The present methods can be used for preventing and/or treating infection by any suitable platelet-targeting bacterium in a subject. In some embodiments, the bacterium can be in a genus of *Staphylococcus*, *Escherichia*, *Streptococcus* or *Helicobacter*. For example, the bacterium can be in the genus of *Staphylococcus*. The *Staphylococcus* bacterium can be in *S. aureus* group (*e.g.*, *S. aureus* or *S. simiae*), *S. auricularis* group (*e.g.*, *S. auricularis*), *S. carnosus* group (*e.g.*, *S. carnosus*, *S. condimenti*, *S. massiliensis*, *S. piscifermentans*, or *S. simulans*), *S. epidermidis* group (*e.g.*, *S. capitis*, *S. caprae*, *S. epidermidis*, or *S. saccharolyticus*), *S. haemolyticus* group (*e.g.*, *S. devriesei*, *S. haemolyticus*, or *S. hominis*), *S. hyicus-intermedius* group (*e.g.*, *S. chromogenes*, *S. felis*, *S. delphini*, *S. hyicus*, *S. intermedius*, *S. lutrae*, *S. microti*, *S. muscae*, *S. pseudintermedius*, *S. rostri*, or *S. schleiferi*), *S. lugdunensis* group (*e.g.*, *S. lugdunensis*), *S. saprophyticus* group (*e.g.*, *S. arlettae*, *S. cohnii*, *S. equorum*, *S. gallinarum*, *S. kloosii*, *S. leei*, *S. nepalensis*, *S. saprophyticus*, *S. succinus*, or *S. xylosus*),

S. sciuri group (*e.g.*, *S. fleurettii*, *S. lentus*, *S. sciuri*, *S. stepanovicii*, or *S. vitulinus*), *S. simulans* group (*e.g.*, *S. simulans*) or *S. warneri* group (*e.g.*, *S. pasteuri*, or *S. warneri*). In another example, the bacterium can be in the genus of *Escherichia*, *e.g.*, *Escherichia coli*. In still another example, the bacterium can be in the genus of *Streptococcus*. The *Streptococcus* bacterium can be *Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus equisimilis, Streptococcus bovis, Streptococcus anginosus, Streptococcus sanguinis, Streptococcus suis, Streptococcus mitis, Streptococcus mutans,* or *Streptococcus pneumoniae.* In yet another example, the bacterium can be in the genus of *Helicobacter*. The *Helicobacter* bacterium can be *Helicobacter pylori* or a non-*pylori Helicobacter* species. The non-*pylori Helicobacter* species can be *H. suis*, *H. baculiformis*, *H. equorum*, *H. hepaticus, H. mustelae*, *H. bilis, H. felis, H. bizzozeronii*, *H. salomonis, H. ganmani*, *H. pullorum, H. anseris*, *H. brantae, H. cinaedi* or *H. canis*.

[000106] In some embodiments, the nanoparticle used in the above combination or pharmaceutical composition can comprise an agent for preventing the bacterial infection, treating the bacterial infection, diagnosing the bacterial infection, prognosing the bacterial infection and/or monitoring prevention or treatment of the bacterial infection. Any suitable agent can be used. In some embodiments, the agent is an antibiotic. Any suitable antibiotic can be used. For example, the antibiotic can have a bactericidal activity or bacteriostatic activity. In another example, the antibiotic can be a narrow-spectrum or a broad-spectrum antibiotic. In still another example, the antibiotic can target the bacterial cell wall (*e.g.*, penicillins and cephalosporins), can target the cell membrane (*e.g.*, polymyxins), can interfere with essential bacterial enzymes (*e.g.*, rifamycins, lipiarmycins, quinolones and sulfonamides), targets protein synthesis (*e.g.*, macrolides, lincosamides and tetracyclines), or can be a cyclic lipopeptide (such as daptomycin), a glycylcycline (such as tigecycline), an oxazolidinone (such as linezolid), or a lipiarmycin (such as fidaxomicin). In other embodiments, the nanoparticle can comprise an agent for decreasing or neutralizing the effect of a bacterial toxin, *e.g.*, a RBC coated nanoparticle. *See e.g.*, U.S. Application Serial No. 13/827,906, filed March 14, 2013, International Application No. PCT/US2012/039411, filed May 24, 2012 and published as WO 2013/052167 A2 and U.S. provisional application Serial No. 61/492,626, filed June 2, 2011.

[000107] In some embodiments, the microbe can be a virus. The above combination or pharmaceutical composition can be used for preventing and/or treating infection by any suitable platelet-targeting virus in a subject. In some embodiments, the virus can be a dsDNA virus (*e.g.*, an adenoviruse, a herpesvirus, a poxvirus), a ssDNA virus (+ strand or "sense") DNA (*e.g.*, a parvovirus), a dsRNA virus (*e.g.*, a reovirus), (+)ssRNA virus (+ strand or sense) RNA (*e.g.*, a picornavirus, a togavirus) or a (-)ssRNA virus (– strand or antisense) RNA (*e.g.*, an orthomyxovirus, a rhabdovirus), a ssRNA-RT virus (+ strand or sense) RNA with DNA intermediate in life-cycle (*e.g.*, a retrovirus) or a dsDNA-RT virus (*e.g.*, a hepadnavirus). In other embodiments, the virus can be an adenovirus (*e.g.*, adenovirus serotypes 1, 2, 4, 5, 6, 7, 8, 14, 19, 36, 37, 40 and 41), a coxsackievirus, a cytomegalovirus (CMV) (*e.g.*, human CMV or human herpesvirus-5 (HHV-5)), a Dengue virus, a hantavirus (*e.g.*, a strain that causes hantavirus hemorrhagic fever with renal syndrome (HFRS) or Hantavirus pulmonary syndrome (HPS)), a hepatitis C virus (HCV), a hepatitis B virus (HBV), a herpesviridae (*e.g.*, Epstein–Barr virus (EBV), also called human herpesvirus 4 (HHV-4)), a lentivirus (*e.g.*, the human immunodeficiency virus (HIV)), a muromegalovirus (*e.g.*, human herpesvirus 6 (HHV-6)), a Roseolovirus (*e.g.*, HHV-6 and human herpesvirus 7 (HHV-7)), or a rotavirus (A, B, C, D, and E rotavirus A).

[000108] In some embodiments, the nanoparticle can comprise an agent, *e.g.*, an anti-viral agent, for preventing the viral infection, treating the viral infection, diagnosing the viral infection, prognosing the viral infection and/or monitoring prevention or treatment of the viral infection. Any suitable anti-viral agent can be used. For example, the anti-viral agent can interfere with the ability of a virus to infiltrate a target cell (*e.g.*, an entry inhibitor and an uncoating inhibitor), can target the processes that synthesize virus components after a virus invades a cell (*e.g.*, an inhibitor or viral reverse transcription, integrase, transcription, translation, protein processing and targeting, or a protease inhibitor), can target viral assembly or can target viral release phase.

[000109] In some embodiments, the microbe can be a fungus. The above combination or pharmaceutical composition can be used for preventing and/or treating infection by any suitable platelet-targeting fungus in a subject. For example, the fungus can be a genus of *Candida* (*e.g.*, *Candida albicans*), *Aspergillus* (*e.g.*, *Aspergillus fumigatus* and *Aspergillus flavus*), *Cryptococcus* (*e.g.*, *Cryptococcus neoformans* and *Cryptococcus gattii*),

Histoplasma (*e.g.*, *Histoplasma capsulatum*), *Mucormycetes* (*e.g.*, *Rhizopus oryzae* (or *Rhizopus microsporus*), *Mucor* species, *Lichtheimia corymbifera*, and *Rhizomucor pusillus*), *Pneumocystis* (*e.g.*, *Pneumocystis jirovecii*), or *Stachybotrys* (*e.g.*, *Stachybotrys chartarum*). In some embodiments, the nanoparticle can comprise an agent, *e.g.*, an anti-fungal agent, for preventing the fungal infection, treating the fungal infection, diagnosing the fungal infection, prognosing the fungal infection and/or monitoring prevention or treatment of the fungal infection. Any suitable anti- fungal agent can be used. For example, the anti-fungal agent can be a polyene antifungal agent, an imidazole antifungal agent, a triazole antifungal agent, a thiazole antifungal agent, an allylamine or an echinocandin.

[000110] In some embodiments, the microbe can be a parasite. The above combination or pharmaceutical composition can be used for preventing and/or treating infection by any suitable platelet-targeting parasite in a subject. For example, the parasite can be in a genus of *Plasmodium* (*e.g.*, *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesii*), *Schistosoma* (*Schistosoma guineensis*, *Schistosoma intercalatum*, *Schistosoma haematobium*, *Schistosoma japonicum*, *Schistosoma malayensis*, *Schistosoma mansoni* and *Schistosoma mekongi*) or *Toxoplasma gondii*. In some embodiments, the nanoparticle can comprise an agent, *e.g.*, an anti-parasitic agent, for preventing the parasitic infection, treating the parasitic infection, diagnosing the parasitic infection, prognosing the parasitic infection and/or monitoring prevention or treatment of the parasitic infection. Any suitable anti-parasitic agent can be used. For example, the anti-parasitic agent can be an anti-worm agent, an anti-malaria agent or an anti-*Toxoplasma gondii* agent. Any suitable anti-malaria agent can be used. For example, the an anti-malaria agent can be used for treating malaria in a subject with suspected or confirmed infection, for preventing infection in a subject, or for routine intermittent treatment of certain groups of subjects in an endemic region. In another example, the anti-malaria agent can be a quinine and related agent, chloroquine, amodiaquine, pyrimethamine, proguanil, a sulfonamide, mefloquine, atovaquone, primaquine, artemisinin and derivative, halofantrine, doxycycline, or clindamycin.

[000111] The above combination or pharmaceutical composition can further comprise another active ingredient to the subject. The other active ingredient can be used for

preventing and/or treating infection by a platelet-targeting microbe in a subject. Any suitable active ingredient can be used. For example, the other active ingredient can comprise a red blood cell membrane coated nanoparticle comprising an inner core comprising a non-cellular material and an outer surface comprising a cellular membrane derived from a red blood cell. The red blood cell membrane coated nanoparticle can be used to decrease or neutralize the effect of a RBC-targeting toxin produced by the microbe in a subject. The red blood cell membrane coated nanoparticle can further comprise active ingredient for preventing and/or treating infection by a microbe in a subject. The red blood cell membrane coated nanoparticle can also comprise active ingredient for preventing and/or treating infection by a RBC-targeting microbe in a subject.

[000112] In one example, the infection to be prevented and/or treated is infection by a platelet-targeting microbe that also produces a RBC-targeting toxin. One such exemplary microbe is *S. aureus*, *e.g.*, methicillin-resistant *Staphylococcus aureus* (MRSA), which produces RBC-targeting alpha-toxin (also known as hemolysin). The present methods can use a combination of a platelet coated nanoparticle, optionally containing an antibiotic, to reduce the microbial count or eliminate the microbe in a subject, and a RBC coated nanoparticle to decrease or neutralize the effect of a toxin produced by the microbe in a subject.

[000113] In some embodiments, the above combination or pharmaceutical composition can further comprise a pharmaceutically acceptable carrier or excipient to the subject.

[000114] The above combination or pharmaceutical composition can be administered using any suitable delivery mechanisms or techniques. In some embodiments, the composition can be administered alone. In other embodiments, the composition can be administered with a pharmaceutically acceptable carrier or excipient. In still other embodiments, the composition can be administered via a medicament delivery system or a medical device. Any suitable medicament delivery system or medical device can be used. For example, the medicament delivery system or the medical device can be an implant, *e.g.*, an implant placed during or after bone surgery, a catheter, or a sustained-release drug delivery system.

[000115] The composition used in the present methods can be administered to the subject via any suitable route of administration. In some embodiments, the nanoparticle used in the present methods, alone or in combination with other active ingredient(s), can be administered via oral, parenteral, rectal, nasal, topical, or ocular routes, or by inhalation. Exemplary parenteral administration can be via intravenous, intramuscular, intraperitoneal, intranasal, or subcutaneous route.

D. Pharmaceutical compositions and administration routes

[000116] The pharmaceutical compositions comprising the nanoparticles, alone or in combination with other active ingredient(s), described herein may further comprise one or more pharmaceutically-acceptable excipients. A pharmaceutically-acceptable excipient is a substance that is non-toxic and otherwise biologically suitable for administration to a subject. Such excipients facilitate administration of the nanoparticles, alone or in combination with other active ingredient(s), described herein and are compatible with the active ingredient. Examples of pharmaceutically-acceptable excipients include stabilizers, lubricants, surfactants, diluents, anti-oxidants, binders, coloring agents, bulking agents, emulsifiers, or taste-modifying agents. In preferred embodiments, pharmaceutical compositions according to the various embodiments are sterile compositions. Pharmaceutical compositions may be prepared using compounding techniques known or that become available to those skilled in the art.

[000117] Sterile compositions are within the present disclosure, including compositions that are in accord with national and local regulations governing such compositions.

[000118] The pharmaceutical compositions and the nanoparticles, alone or in combination with other active ingredient(s), described herein may be formulated as solutions, emulsions, suspensions, or dispersions in suitable pharmaceutical solvents or carriers, or as pills, tablets, lozenges, suppositories, sachets, dragees, granules, powders, powders for reconstitution, or capsules along with solid carriers according to conventional methods known in the art for preparation of various dosage forms. The nanoparticles, alone or in combination with other active ingredient(s), described herein, and preferably in the form of a pharmaceutical composition, may be administered by a suitable route of delivery, such as oral, parenteral, rectal, nasal, topical, or ocular routes, or by inhalation.

In some embodiments, the compositions are formulated for intravenous or oral administration.

[000119] For oral administration, the nanoparticles, alone or in combination with another active ingredient, may be provided in a solid form, such as a tablet or capsule, or as a solution, emulsion, or suspension. To prepare the oral compositions, the nanoparticles, alone or in combination with other active ingredient(s), may be formulated to yield a dosage of, *e.g.*, from about 0.01 to about 50 mg/kg daily, or from about 0.05 to about 20 mg/kg daily, or from about 0.1 to about 10 mg/kg daily. Oral tablets may include the active ingredient(s) mixed with compatible pharmaceutically acceptable excipients such as diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservative agents. Suitable inert fillers include sodium and calcium carbonate, sodium and calcium phosphate, lactose, starch, sugar, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol, and the like. Exemplary liquid oral excipients include ethanol, glycerol, water, and the like. Starch, polyvinyl-pyrrolidone (PVP), sodium starch glycolate, microcrystalline cellulose, and alginic acid are exemplary disintegrating agents. Binding agents may include starch and gelatin. The lubricating agent, if present, may be magnesium stearate, stearic acid, or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate to delay absorption in the gastrointestinal tract, or may be coated with an enteric coating.

[000120] Capsules for oral administration include hard and soft gelatin capsules. To prepare hard gelatin capsules, active ingredient(s) may be mixed with a solid, semi-solid, or liquid diluent. Soft gelatin capsules may be prepared by mixing the active ingredient with water, an oil, such as peanut oil or olive oil, liquid paraffin, a mixture of mono and di-glycerides of short chain fatty acids, polyethylene glycol 400, or propylene glycol.

[000121] Liquids for oral administration may be in the form of suspensions, solutions, emulsions, or syrups, or may be lyophilized or presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid compositions may optionally contain: pharmaceutically-acceptable excipients such as suspending agents (for example, sorbitol, methyl cellulose, sodium alginate, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminum stearate gel and the like); non-aqueous vehicles, e.g.,

oil (for example, almond oil or fractionated coconut oil), propylene glycol, ethyl alcohol, or water; preservatives (for example, methyl or propyl p-hydroxybenzoate or sorbic acid); wetting agents such as lecithin; and, if desired, flavoring or coloring agents.

[000122] The compositions may be formulated for rectal administration as a suppository. For parenteral use, including intravenous, intramuscular, intraperitoneal, intranasal, or subcutaneous routes, the nanoparticles, alone or in combination with other active ingredient(s), may be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity or in parenterally acceptable oil. Suitable aqueous vehicles can include Ringer's solution and isotonic sodium chloride. Such forms may be presented in unit-dose form such as ampoules or disposable injection devices, in multi-dose forms such as vials from which the appropriate dose may be withdrawn, or in a solid form or pre-concentrate that can be used to prepare an injectable formulation. Illustrative infusion doses range from about 1 to 1000 μg/kg/minute of agent admixed with a pharmaceutical carrier over a period ranging from several minutes to several days.

[000123] For nasal, inhaled, or oral administration, the nanoparticles, alone or in combination with other active ingredient(s), may be administered using, for example, a spray formulation also containing a suitable carrier.

[000124] For topical applications, the nanoparticles, alone or in combination with other active ingredient(s), are preferably formulated as creams or ointments or a similar vehicle suitable for topical administration. For topical administration, the nanoparticles, alone or in combination with other active ingredient(s), may be mixed with a pharmaceutical carrier at a concentration of about 0.1% to about 10% of drug to vehicle. Another mode of administering the nanoparticles, alone or in combination with other active ingredient(s), may utilize a patch formulation to effect transdermal delivery.

[000125] In certain embodiments, the present disclosure provides a pharmaceutical composition comprising the nanoparticles, alone or in combination with other active ingredient(s), and methylcellulose. In certain embodiments, methylcellulose is in a suspension of about 0.1, 0.2, 0.3, 0.4, or 0.5 to about 1%. In certain embodiments, methylcellulose is in a suspension of about 0.1 to about 0.5, 0.6, 0.7, 0.8, 0.9, or 1%. In certain embodiments, methylcellulose is in a suspension of about 0.1 to about 1%. In

certain embodiments, methylcellulose is in a suspension of about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.8, or 1%. In certain embodiments, methylcellulose is in a suspension of about 0.5%.

E. Example 1. Nanoparticle biointerfacing via platelet membrane cloaking

[000126] One aspect of the invention relates to the preparation of polymeric nanoparticles enclosed in the plasma membrane of human platelets, which are a unique population of cellular fragments that adhere to a variety of disease-relevant substrates[4-7]. The resulting nanoparticles possess a right-side-out unilamellar membrane coating functionalized with immunomodulatory and adhesion antigens associated with platelets. As compared to uncoated particles, the platelet membrane-cloaked nanoparticles have reduced cellular uptake by macrophage-like cells and are absent of particle-induced complement activation in autologous human plasma. The cloaked nanoparticles also display platelet-mimicking properties as well as enhanced binding to platelet-adhering pathogens. In a mouse model of systemic bacterial infection, docetaxel and vancomycin, respectively, show enhanced therapeutic efficacy when delivered by the platelet-mimetic nanoparticles. The multifaceted biointerfacing enabled by the platelet membrane cloaking method provides a new approach in developing functional nanoparticles for disease-targeted delivery.

[000127] Owing to their role as circulating sentinels for vascular damage as well as for invasive microorganisms, platelets have inspired the design of many functional nanocarriers[8-13]. The multitude of platelet functions stem from a unique set of surface moieties responsible for immune evasion[14,15], subendothelium adhesion[5,16], and pathogen interactions[6,7]. By adopting a recently developed cell membrane cloaking technique[17-19], we demonstrate the preparation of platelet membrane-cloaked nanoparticles (PNPs) consisting of a biodegradable polymeric nanoparticle core shielded entirely in the plasma membrane of human platelets. Several inherent platelet properties, including immunocompatibility, binding to injured vasculature, and pathogen adhesion, as well as their therapeutic implications, were studied (Figure 4A).

[000128] PNPs were prepared by fusing plasma membranes derived from human platelets with 100 nm poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles. Platelet-rich

plasma collected from human blood was first mixed with EDTA, which prevents platelet aggregation by deactivating fibrinogen-binding integrin αIIbβ3[20]. Platelets were then processed for nanoparticle membrane cloaking (Figure 4B). Physicochemical characterizations revealed that the final PNPs were approximately 15 nm larger than the uncoated PLGA nanoparticles (bare NPs) and possessed an equivalent surface charge to that of platelet and platelet membrane-derived vesicles (platelet vesicles) (Figure 1A). Transmission electron microscopy (TEM) visualization of platelet vesicles and PNPs showed the formation of distinctive nanoparticulates and consistent unilamellar membrane coatings over the polymeric cores (Figure 1B and figures 5A-5H). Improved colloidal stability was observed with the PNPs compared to bare NPs (Figure 1C), which is attributable to the stabilizing effect by the plasma membrane's hydrophilic surface glycans[21]. Translocation of platelet membrane protein content, including immunomodulatory proteins, CD47, CD55, and CD59[14,15], integrin components, αIIbβ3, α2, α5, α6, and β1, and other transmembrane proteins, GPIbα, GPIV, GPV, GPVI, GPIX, and CLEC-2[5,16], onto the nanoparticles was verified via western blotting (Figure 1D and Figure 6). The majority of the membrane proteins were largely retained with the exception of GPIbα and GPV, which showed noticeable alterations attributable to their susceptibility to protease cleavage upon platelet perturbation[22]. A right-side-out membrane orientation on the PNPs was verified using both immunogold staining and flow cytometric analysis with antibodies targeting either the intracellular or extracellular domain of CD47 (Figure 1E and Figures 7A-7D). Pro-thrombotic, platelet-activating molecules such as thrombin, ADP, and thromboxane were removed in the PNP formulation (Figures 1F-1H), thereby permitting PNP administration with little risk of a thrombotic response (Figigure 1I).

[000129] PNPs' platelet-mimicking functionalities were first studied via the particles' binding to human type IV collagen, a primary subendothelial component[23]. Fluorescently labeled PNPs, along with bare NPs and red blood cell membrane-cloaked nanoparticles (RBCNPs), were incubated on collagen-coated plates. The platelet membrane cloak significantly enhanced particle retention as compared to bare NPs and RBCNPs (Figure 2A), indicating that the collagen adhesion was membrane type specific. Reduced PNP retention on non-collagen coated plates and in the presence of anti-GPVI antibodies supports a specific collagen/platelet membrane interaction attributable to the presence of membrane glycoprotein receptors for collagen[16] (Figure 6). Further examination of PNP's

differential binding to endothelial and collagen surfaces was performed using collagen-coated tissue culture slides seeded with human umbilical vein endothelial cells (HUVECs). PNPs adhered primarily outside of areas encompassed by the cells (Figure 2B and Figures 8A-8G). In addition, the PNPs were incubated with the extracellular matrix derived from decellularized human umbilical cord arteries. Following PBS washes, scanning electron microscopy (SEM) revealed a significant number of PNPs remaining on the fibrous structures on the luminal side of the artery (Figure 2C and Figures 8H-8I).

[000130] Examination of PNPs' immunocompatibility was conducted using differentiated human THP-1 macrophage-like cells. The platelet membrane cloaking reduced particle internalization in a CD47-specific manner[24], as blocking by anti-CD47 antibodies increased the cellular uptake (Figure 2D). The PNPs were further investigated for their interactions with the complement system based on quantifications of C4d and Bb split products. Following incubation in human plasma, complement activation was observed with bare NPs, reflecting their susceptibility to opsonization as well as the spontaneous reaction between C3 thioesters and the hydroxyl groups on the PLGA particles[25]. In contrast, an equal amount of PNPs mixed with autologous plasma showed no observable complement activation (Figures 2E-2F). This suppression of the complement system can be attributed to membrane-bound complement regulator proteins such as CD55 and CD59[26] (Figure 6). The lack of complement activation by the PNPs also attests to the completeness of the membrane cloaking, which shields the polymeric cores from plasma exposure and minimizes the risk of anaphylatoxin generation frequently associated with injectable nanocarriers[27].

[000131] The therapeutic potential of PNPs against platelet-adhering pathogens was further examined. Opportunistic bacteria including several strains of staphylococci and streptococci exploit platelets via both direct and indirect adherence mechanisms for tissue localization and immune evasion[6]. To demonstrate that PNPs can exploit the inherent bacterial adherence mechanism for targeted antibiotics delivery, MRSA252, a strain of methicillin-resistant *Staphylococcus aureus* expressing a serine-rich adhesin for platelets (SraP)[28], was used as a model pathogen for particle adhesion study. Following 10 min of incubation between formalin-fixed MRSA252 and different nanoformulations, the collected bacteria showed preferential binding by PNPs (Figure 3A), exhibiting a 12-fold

increase in PNP retention as compared to bare NPs (Figure 3B and Figures 10A-10C). This adherence was membrane-specific as RBCNPs showed lower retention than PNPs. The therapeutic potential of PNPs was further evaluated using vancomycin-loaded formulations. In an *in vitro* antimicrobial study, live MRSA252 bacteria were briefly incubated with free vancomycin, vancomycin-loaded RBCNPs (RBCNP-Vanc), or vancomycin-loaded PNPs (PNP-Vanc) followed by a subsequent wash and culturing in fresh media. The PNP-Vanc formulation showed statistically significant improvement in MRSA252 reduction that corroborates the targeting effect of the particles (Figure 3C). An *in vivo* antimicrobial efficacy study was further conducted using a mouse model of systemic MRSA252 infection. Mice systemically challenged with 6×10^6 CFU MRSA252 received once daily intravenous treatment of free vancomycin, RBCNP-Vanc, or PNP-Vanc for 3 days at 10 mg kg^{-1} of vancomycin. A control group of high-dose vancomycin treatment in which infected mice received a therapeutic concentration of vancomycin at 30 mg kg^{-1} twice daily was conducted in parallel. 24 h following the last treatment, bacterial enumeration at the primary infection organs showed that the PNP-Vanc resulted in the lowest mean bacterial counts across all organs (Figures 3D-3I). Statistical analyses revealed significance between PNP-Vanc and free vancomycin at equivalent dosage in the lung, liver, spleen, and kidney. In comparison to free vancomycin at 6-fold the dosage, PNP-Vanc showed significantly better antimicrobial efficacy in the liver and spleen while being at least equally effective in the blood, heart, lung, and kidney. Notably, as compared to RBCNP-Vanc, PNP-Vanc showed significantly higher potency in the heart, lung, liver, and spleen, reflecting membrane-specific modulation of nanoparticle performance. The study validates the feasibility of harnessing biomembrane interfaces to improve infectious disease treatment.

[000132] The vast medical relevance of platelets has inspired many platelet-mimicking systems that target dysfunctional vasculature in cardiovascular diseases[8,9], traumas[10,11,13], cancers[12], and acute inflammations[29]. The present PNP platform exploits platelet membrane in its entirety to enable biomimetic interactions with proteins, cells, tissues, and microorganisms. Toward translation, the platform would benefit from existing infrastructures and logistics for transfusion medicine, polymeric nanotherapeutics, and cell-derived pharmaceutics. Prior works on the cell membrane cloaking approach demonstrated high cloaking efficiency[30] and viable storage[18] upon platform optimization

(Figures 5F-5H). By employing large-scale purification and dispersion techniques commonly applied to biologics, reliable platelet membrane derivation and PNP production can be envisioned.

Method Summary

[000133] Platelets were collected from human whole blood via centrifugal separation, and membrane vesicles were derived from the cells through a combination of freeze-thaw cycles, washing, and sonication. PNPs were prepared by fusing platelet vesicles onto preformed PLGA nanoparticles via ultrasonic dispersion. Drug-loaded PNPs were prepared by cloaking drug-loaded nanoparticle cores with platelet membranes. Size and surface zeta potential of PNPs were obtained from DLS measurements. Core-shell structure of PNPs was examined by TEM. PNPs were characterized for protein retention using SDS-PAGE, and specific immunomodulatory and adhesion membrane proteins were subsequently identified using western blotting. Membrane sidedness was examined based on the orientation of CD47 transmembrane proteins on the PNPs.

[000134] Differential binding of PNPs to subendothelium was assessed based on particle retention on human type IV collagen-coated plates seeded with HUVECs and to subendothelial matrices of decellularized human umbilical cord arteries. Immunocompatibility of PNPs was assessed through uptake by differentiated THP-1 cells and through both classical and alternative complement activation. Adherence to damaged vasculatures was examined through an *ex vivo* study with denuded human carotid arteries and an *in vivo* study with rats following angioplasty-induced endothelial denudation. Therapeutic efficacy of docetaxel-loaded PNPs was assessed in an experimental rat model of coronary restenosis.

Methods

[000135] ***Human platelet isolation and platelet membrane derivation*:** Fresh type O^- human blood with EDTA as an anticoagulant was purchased from BioreclamationIVT. To isolate platelets from whole blood, the blood sample was first centrifuged at 100 × g for 20 min at room temperature to separate red blood cells and white blood cells from platelet rich plasma (PRP). The PRP was then centrifuged at 100 × g for 20 min to remove remaining blood cells. PBS buffer containing 1 mM of EDTA and 2 μM of prostaglandin

E1 (PGE1, Sigma Aldrich) was added to the purified PRP to prevent platelet activation. Platelets were then pelleted by centrifugation at 800 × g for 20 min at room temperature, following which the supernatant was discarded and the platelets were resuspended in PBS mixed with protease inhibitor tablets (Pierce). 1.5 mL aliquots of platelet solution containing ~ 3 x 10^9 platelets were used to cloak 1 mg of PLGA nanoparticles.

[000136] Platelet membrane was derived by a repeated freeze-thaw process. Aliquots of platelet suspensions were first frozen at -80 °C, thawed at room temperature, and pelleted by centrifugation at 4000 × g for 3 min. Following three repeated washes with PBS solution mixed with protease inhibitor tablets, the pelleted platelet membranes were suspended in water and sonicated in a capped glass vial for 5 min using a Fisher Scientific FS30D bath sonicator at a frequency of 42 kHz and a power of 100 W. The presence of platelet membrane vesicles was verified by size measurement using dynamic light scattering (DLS) and morphological examination by transmission electron microscopy (TEM).

[000137] ***Platelet membrane-cloaked nanoparticle (PNP) preparation and characterization*:** 100 nm polymeric cores were prepared using 0.67 dL g^{-1} carboxyl-terminated 50:50 poly(lactic-*co*-glycolic) acid (PLGA) (LACTEL Absorbable Polymers) in a nanoprecipitation process. 1 mL of 10 mg mL^{-1} PLGA solution in acetone was added dropwise to 3 mL of water. For fluorescently labeled nanoformulations, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate (DiD, ex = 644 nm/em = 665 nm, Life Technologies) was loaded into the polymeric cores at 0.1 wt%. The mixture was then stirred in open air for 1 h and placed in vacuum for another 3 h. The resulting nanoparticle solution was filtered with 10 kDa MWCO Amicon Ultra-4 Centrifugal Filters (Millipore). Platelet membrane cloaking was then accomplished by dispersing and fusing platelet membrane vesicles with PLGA particles via sonication using an FS30D bath sonicator at a frequency of 42 kHz and a power of 100 W for 2 min. The size and the surface zeta potential of the resulting PNPs were obtained by DLS measurements using a Malvern ZEN 3600 Zetasizer. PBS stability was examined by mixing 1 mg mL^{-1} of PNPs in water with 2X PBS at a 1:1 volume ratio. Storability of PNPs was examined by suspending PNPs in 10% sucrose. The nanoparticle solutions were subject to either a freeze-thaw cycle or lyophilization followed by resuspension. The resulting particle solution was then

monitored for particle size using DLS. The structure of PNPs was examined with TEM following negative staining with 1 wt% uranyl acetate using an FEI 200 kV Sphera microscope. RBCNPs were prepared using the same polymeric cores and RBC membranes of equivalent total surface area to the platelet membranes using a previously described protocol[16]. The RBCNPs were characterized using DLS and had similar size and zeta potential as the PNPs.

[000138] Docetaxel-loaded PLGA nanoparticle cores were prepared via a nanoprecipitation process. 10 wt% docetaxel was added to 5 mg PLGA in acetone and precipitated dropwise into 3 mL water. The solvent was evaporated as described above and free docetaxel was removed via repeated wash steps. Vancomycin-loaded nanoparticles were synthesized using a double emulsion process. The inner aqueous phase consisted of 25 μL of vancomycin (Sigma Aldrich) dissolved in 1 M NaOH at 200 mg mL^{-1}. The outer phase consisted of 500 μL of PLGA polymer dissolved in dichloromethane at 50 mg mL^{-1}. The first emulsion was formed via sonication at 70% power pulsed (2 sec on/1 sec off) for 2 min on a Fisher Scientific 150E Sonic Dismembrator. The resulting emulsion was then emulsified in aqueous solution under the same dispersion setting. The final w/o/w emulsion was added to 10 mL of water and the solvent was evaporated in a fume food under gentle stirring for 3 h. The particles were collected via centrifugation at 80,000 × g in a Beckman Coulter Optima L-90K Ultracentrifuge. The particles were washed and resuspended in water. Upon preparation of drug-loaded PLGA cores, cell membrane coating was performed by adding the appropriate surface area equivalent of either platelet or RBC membrane followed by 3 min of sonication in a Fisher Scientific FS30D Bath Sonicator. Particle size, polydispersity (PDI), and surface zeta potential were characterized using DLS. Drug loading yield and release rate were quantified by high performance liquid chromatography (HPLC). Drug release was determined by dialyzing 500 μL of particle solution at a concentration of 2.67 mg mL^{-1} in PBS using 3.5K MWCO Slide-A-Lyzers (Thermo Scientific).

[000139] ***Examination of platelet membrane proteins:*** PNPs were purified from unbound proteins or membrane fragments via centrifugation at 16,000 × g in 10% sucrose. Platelet-rich plasma, platelets, platelet membrane vesicles, and PNPs were then normalized to equivalent overall protein concentration using a Pierce BCA Protein Assay

Kit (Life Technologies). The samples containing equivalent total proteins were then lyophilized, prepared in lithium dodecyl sulfate (LDS) sample loading buffer (Invitrogen), and separated on a 4-12% Bis-Tris 10-well minigel in MOPS running buffer using a Novex Xcell SureLock Electrophoresis System (Life Technologies). For membrane protein visualization, the gel was stained using SimplyBlue SafeStain solution (Life Technologies) following the manufacturer's instructions and imaged using a gel imager. Identification of key membrane proteins via western blotting was performed using primary antibodies including mouse anti-human CD47 (Biolegend, CC2C6), mouse anti-human CD55 (Biolegend, JS11), mouse anti-human CD59 (Biolegend, H19), mouse anti-human integrin αIIbβ3 (Biolegend, PAC-1), mouse anti-human integrin α2 subunit (Abgent, AP17907b-ev), mouse anti-human integrin α5 subunit (Biolegend, NKI-SAM-1), mouse anti-human integrin α6 subunit (Biolegend, GoH3), mouse anti-human integrin β1 subunit (Biolegend, TS2/16), mouse anti-human GPIbα (Biolegend, HIP1), mouse anti-human GPIV (Biolegend, 5-271), rabbit anti-human GPV (Santa Cruz Biotech, H-300), rabbit anti-human GPVI (Santa Cruz Biotech, H-5), rabbit anti-human GPIX (Santa Cruz Biotech, A-9), and mouse anti-human CLEC-2 (Santa Cruz Biotech, 53Ex9). A goat anti-mouse IgG-HRP conjugate (Biolegend, Poly4053) and a donkey anti-rabbit IgG-HRP conjugate (Biolegend, Poly4064) were used for secondary staining based on the isotype of the primary antibodies. The nitrocellulose membrane was then incubated with ECL western blotting substrate (Pierce) and developed with the Mini-Medical/90 Developer (ImageWorks).

[000140] ***Examination of protein sidedness on PNPs:*** For immunogold staining, a drop of the PNP solution (1 mg mL^{-1}) was deposited onto a glow-discharged carbon-coated grid. The grid was then washed 3 times with PBS, blocked with 1% BSA for 15 min, and stained with 0.5 mg mL^{-1} of anti-CD47 targeted to either the intracellular or extracellular domain of the protein. Following 1 h of incubation, the samples were rinsed with PBS containing 1% BSA for 6 times and stained with anti-rabbit IgG gold conjugate (5 nm) solution (Sigma Aldrich) for another hour. Following 6 times of PBS washing, the samples were fixed with 1% glutaraldehyde in PBS for 5 min and washed with water for 6 times. The sample grids were subsequently stained with 2% vanadium solution (Abcam) and visualized using an FEI 200 kV Sphera microscope.

[000141] For flow cytometric analysis, 2.0 μm carboxyl-functionalized polystyrene beads at a concentration of 4 wt% (Life Technologies) were functionalized with rabbit N-terminus-targeted (extracellular) anti-human CD47 (Aviva Biosystems, ARP63284), rabbit intracellular-domain-targeted anti-CD47 (Genetex, EPR4150(2)), or rabbit anti-ovalbumin (Abcam, ab1221) as a sham antibody via EDC/NHS chemistry. The resulting antibody-modified beads were re-suspended in 100 μL of DI water. The bead solution was first incubated with 1 mg of bovine serum albumin (BSA, Sigma Aldrich) to block non-specific interactions and then mixed with 1 mL of fluorescently labeled PNPs (200 μg mL^{-1}). The mixture solution was incubated at room temperature for 2 h and then centrifuged to remove the unbound PNPs. The collected polystyrene beads were then subjected to flow cytometric analysis.

[000142] ***Platelet aggregation assay*:** Platelets, platelet membrane vesicles, and PNPs of equivalent membrane content were prepared and examined for platelet-activating molecules, including thrombin, ADP, and thromboxane, using a SensoLyte 520 Thrombin Activity Assay Kit (AnaSpec), ADP Colorimetric/Fluorometric Assay Kit (Sigma Aldrich), and Thromboxane B2 (TXB_2) ELISA Kit (Enzo Life Sciences), respectively, based on the manufacturers' instructions.

[000143] Aggregation of platelets in the presence of PNPs was assessed using a spectrophotometric method. 1 mL aliquot of platelet rich plasma (PRP) was first prepared from human whole blood with sodium citrate as the anti-coagulant. The plasma was then loaded into a cuvette followed by addition of 500 μL of 2 mg mL^{-1} PNPs in PBS solution. As negative and positive controls, the PRP was mixed with 500 μL of PBS or 500 μL of PBS containing 0.5 IU mL^{-1} of human thrombin (Sigma Aldrich), respectively. The cuvettes were immediately placed in a TeCan Infinite M200 reader and monitored for change in absorbance at 650 nm over time, and platelet aggregation was observed based on the reduction of turbidity.

[000144] ***Collagen binding study*:** Collagen type IV derived from human placenta (Sigma Aldrich) was reconstituted to a concentration of 2.0 mg mL^{-1} in 0.25% acetic acid. 200 μL of the collagen solution was then added to a 96-well assay plate and incubated overnight at 4 °C. Prior to the collagen binding study, the plate was blocked with 2% BSA and washed three times with PBS. For the collagen binding study, 100 μL of 1 mg mL^{-1}

DiD-loaded nanoformulations in water were added to the collagen-coated and non-collagen-coated plates. Following 30 sec of incubation, the plates were washed three times. Retained nanoparticles were then dissolved with 100 μL of DMSO for fluorescence quantification using a TeCan Infinite M200 reader.

[000145] ***Differential adhesion to endothelial and collagen surfaces*:** Collagen type IV was coated on 8-well Lab-Tek II chamber slides (Nunclon) as described above. The collagen-coated chamber slides were used to seed HUVECs (American Type Culture Collection) and cultured in HUVEC Culture Medium (Sigma Aldrich) supplemented with 10% fetal bovine serum for 24 h. The cells were then incubated with 1 mg mL^{-1} DiD-loaded PNPs in PBS at 4°C for 30 sec. Next the cells were washed with PBS three times and fixed with tissue fixative (Millipore) for 30 min at room temperature. Fluorescence staining was done with 4'6-diamidino-2-phenylinodle (DAPI, Life Technologies) for the nuclei and 22-(n-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-23,24-bisnor-5-cholen-3β-ol (NBD cholesterol, Life Technologies) for the cytosol before mounting the cells in ProLong Gold antifade reagent (Life Technologies) and imaged using a DeltaVision deconvolution scanning fluorescence microscope. Z-stacks were collected at 0.25 μm intervals over 10 μm. The images were deconvolved and superimposed. DiD fluorescence signal over collagen and endothelial surfaces as defined by the boundaries of NBD fluorescence were analyzed using ImageJ. PNP retention over collagen and endothelial surfaces was quantified based on 10 distinct images in which the average fluorescence per unit area was analyzed.

[000146] ***Cellular uptake study with macrophage-like cells***: THP-1 cells (American Type Culture Collection) were maintained in RPMI 1640 media (Life Technologies) supplemented with 10% FBS (Sigma Aldrich). THP-1 cells were differentiated in 100 ng mL^{-1} phorbol myristate acetate (PMA, Sigma Aldrich) for 48 h and differentiation was visually confirmed by cellular attachment to petri dishes. For the cellular uptake study, the differentiated macrophage-like cells were incubated with DiD-loaded PNPs, anti-CD47 blocked PNPs, and bare NPs at 100 μg mL^{-1} in culture media. Following 30 min of incubation at 37 °C, the macrophage-like cells were scraped off the petri dish and washed three times in PBS to remove non-internalized particles. Flow cytometry was performed to examine nanoparticle uptake by the macrophage-like cells. All flow cytometry studies

were conducted on a FACSCanto II flow cytometer (BD Biosciences) and the data was analyzed using FlowJo software from Tree Star. The mean fluorescence was plotted in a bar chart with error bars representing the standard error. Statistical analysis was performed based on a two-tailed, unpaired t-test.

[000147] ***Complement activation study:*** To assess complement system activation, two complement split products (C4d and Bb) were analyzed using enzyme-linked immunosorbent assay kits (Quidel Corporation). The nanoparticles were incubated with human serum at a volume ratio of 1:5 in a shaking incubator (80 rpm) at 37 °C for 1 h. The reaction was then stopped by adding 60 volumes of PBS containing 0.05% Tween-20 and 0.035% ProClin 300. Complement system activation of the nanoparticles was assayed following the manufacturer's instructions, and zymosan was used as a positive control.

[000148] ***Staphylococcus aureus (MRSA252) bacteria adherence study:*** MRSA252 (American Type Culture Collection) was cultured on tryptic soy broth (TSB) agar (Becton, Dickinson and Company) overnight at 37 °C. A single colony was inoculated in TSB medium at 37 °C in a rotary shaker. Overnight culture was refreshed in TSB medium at 1:100 dilution at 37 °C under shaking for another 3 h until OD_{600} of the culture medium reached approximately 1.0 (logarithmic growth phase). The bacteria were harvested by centrifugation at 5,000 × g for 10 min and then washed with sterile PBS twice and then fixed with 10% formalin for 1 h. The fixed bacteria were washed with sterile PBS and suspended in 10% sucrose to a concentration of 1×10^8 CFU mL^{-1}. For the nanoparticle adhesion study, aliquots of 0.8 mL of 1×10^8 CFU mL^{-1} MRSA252 were mixed with 1.2 mL of 200 μg mL^{-1} DiD-loaded PNPs, RBCNPs, or bare NPs in 10% sucrose for 10 min at room temperature. The bacteria were then isolated from unbound nanoparticles by repeated centrifugal washes in sucrose solution at 5,000 × g. The purified bacteria were then suspended in 10% sucrose for flow cytometric analysis and SEM imaging.

[000149] ***Antimicrobial efficacy study*:** For *in vitro* antimicrobial efficacy study, 5×10^6 CFU of MRSA252 was mixed with 500 μL of 20 mg mL^{-1} nanoparticles (4 wt% vancomycin loading) in saline. As controls, equivalent amount of bacteria was incubated in either PBS or free vancomycin (0.8 mg mL^{-1}). Following 10 min of incubation, bacteria were isolated from the solution via centrifugation at 2500 × g for 5 min. The collected bacteria pellet was resuspended with 500 μL of TSB culture medium and incubated for 5

h. The resulting samples were serially diluted in PBS and spotted on TSB agar plates. Following 24 h of culturing, the colonies were counted to determine the bacteria count in each sample.

[000150] For *in vivo* antimicrobial efficacy study, vancomycin-loaded PNPs (PNP-Vanc) and vancomycin-loaded RBCNPs (RBCNP-Vanc) were suspended in 10% sucrose solution at 31.25 mg mL^{-1} (4 wt% vancomycin loading). An equivalent concentration of free vancomycin (1.25 mg mL^{-1}) was also suspended in 10% sucrose. Male CD-1 mice (Harlan Laboratories) weighing ~25 g were challenged intravenously with 6 x 10^6 CFU of MRSA252 suspended in 100 μL of PBS. 30 min following the bacteria injection, mice were injected with 200 μL of PNP-Vanc, RBCNP-Vanc, free vancomycin (daily dosage: 10 mg kg^{-1} vancomycin), or PBS. To compare to the clinical dosing of vancomycin, a control group treated with twice daily dosing of 30 mg kg^{-1} free vancomycin was prepared (total daily dosage: 60 mg kg^{-1} vancomycin). The mice received their corresponding treatments from day 0 to 2. On day 3, blood was collected from the submandibular vein. The mice were then sacrificed, perfused with PBS, and their organs were collected. The organs were homogenized using a Biospec Mini Beadbeater for 1 min, serially diluted in PBS, and plated onto agar plates. Following 48 h of culture, bacterial colonies were counted to determine the bacterial load in each organ. Data points on the X-axis represent samples with no detectable bacterial colony. It was confirmed that samples prepared from unchallenged mice had no detectable colonies. The data was tested for normal distribution via the Shapiro-Wilk test. For blood and heart, which contained non-normal distributions, statistical analysis was performed using Kruskal-Wallis test. For the other organs, in which all groups were normally distributed, statistical analysis was performed using one-way ANOVA

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CLAIMS

1. A method for preventing and/or treating infection by a platelet-targeting microbe in a subject, which method comprises administering, to a subject in need, or to cells of said subject, an effective amount of a nanoparticle comprising a) an inner core comprising a non-cellular material, b) an outer surface comprising a cellular membrane derived from a platelet; and optionally c) an agent for preventing said infection, treating said infection, diagnosing said infection, prognosing said infection and/or monitoring prevention or treatment of said infection.

2. The method of claim 1, wherein the subject is a mammal.

3. The method of claim 2, wherein the mammal is a human.

4. The method of claim 2, wherein the mammal is a non-human mammal.

5. The method of any of the claims 1-4, which is used for preventing the infection in a subject.

6. The method of any of the claims 1-4, which is used for treating the infection in a subject.

7. The method of any of the claims 1-6, wherein the inner core comprises a polymeric particle core.

8. The method of claim 7, wherein the polymeric particle core comprises an optical shift property.

9. The method of claim 7, wherein the polymeric particle core comprises metal.

10. The method of claim 9, wherein the metal is gold, iron oxide or a quantum dot.

11. The method of any of the claims 1-10, wherein the inner core comprises a biocompatible or a synthetic material selected from the group consisting of poly(lactic-c -glycolic acid) (PLGA), polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL), polylysine, and polyglutamic acid.

12. The method of any of the claims 1-11, wherein the inner core supports the outer surface.

13. The method of any of the claims 1-12, wherein the cellular membrane comprises a plasma membrane derived from the platelet.

14. The method of any of the claims 1-13, wherein the nanoparticle further comprises a releasable cargo.

15. The method of claim 14, wherein the releasable cargo is located within or on the inner core, between the inner core and the outer surface, or within or on the outer surface.

16. The method of claim 14 or 15, wherein the release of the releasable cargo is triggered by a contact between the nanoparticle and the subject or cells of the subject, or by a change of a physical parameter surrounding the nanoparticle.

17. The method of any of the claims 14-16, wherein the releasable cargo is a therapeutic agent, a prophylactic agent, a diagnostic or marker agent, a prognostic agent, an isolation agent, a monitoring agent, or a combination thereof.

18. The method of claim 17, wherein the therapeutic agent is used for treating the infection.

19. The method of claim 17, wherein the prophylactic agent is used for preventing the infection.

20. The method of claim 17, wherein the diagnostic or marker agent is used for diagnosing the infection.

21. The method of claim 17, wherein the prognosing or marker agent is used for prognostic the infection.

22. The method of claim 17, wherein the monitoring agent is used for monitoring prevention or treatment of the infection.

23. The method of claim 17, wherein the isolation agent is used for facilitating isolation and removal of a cell, tissue or organ associated with the infection from the subject.

24. The method of claim 23, wherein the isolation agent comprises a magnetic material.

25. The method of claim 24, wherein the magnetic material comprises iron oxide.

26. The method of any of the claims 14-25, wherein the releasable cargo is a metallic particle, a polymeric particle, a dendrimer particle, or an inorganic particle.

27. The method of any of the claims 1-26, wherein the nanoparticle has a diameter from about 10 nm to about 10 μm.

28. The method of any of the claims 1-27, wherein the nanoparticle substantially lacks constituents of the platelet from which the cellular membrane is derived.

29. The method of any of the claims 1-28, wherein the nanoparticle substantially maintains natural structural integrity or activity of the cellular membrane derived from the platelet or the constituents of the cellular membrane derived from the platelet.

30. The method of any of the claims 1-29, wherein the nanoparticle is biocompatible or biodegradable.

31. The method of claim 1, wherein the inner core of the nanoparticle comprises PLGA and the outer surface of the nanoparticle comprises a plasma membrane derived from the platelet.

32. The method of any of the claims 1-31, wherein the outer surface of the nanoparticle further comprises a synthetic membrane.

33. The method of claim 32, wherein the outer surface of the nanoparticle comprises a hybrid membrane comprising a cellular membrane derived from a platelet and a synthetic membrane.

34. The method of any of the claims 1-33, wherein the nanoparticle substantially lacks immunogenicity to the subject.

35. The method of claim 34, wherein the cellular membrane is derived from a platelet from the same species of the subject.

36. The method of claim 35, wherein the subject is a human and the cellular membrane is derived from a human platelet.

37. The method of claim 36, wherein the cellular membrane is derived from a platelet of the human to be treated.

38. The method of any of the claims 1-37, which is used to reduce the microbial count or eliminate the microbe in a subject, to decrease or neutralize the effect of a toxin produced by the microbe in a subject, and/or to decrease or neutralize a subject's response to the microbe or the microbial toxin in the subject.

39. The method of claim 38, which is used to reduce the microbial count or eliminate the microbe in a subject.

40. The method of claim 38, which is used to decrease or neutralize the effect of a toxin produced by the microbe in a subject.

41. The method of claim 38, which is used to decrease or neutralize a subject's response to the microbe or the microbial toxin in the subject.

42. The method of claim 41, wherein the subject's response to the microbe or the microbial toxin has harmful effect on the subject, *e.g.*, contributing to tissue damage of the subject, supporting microbial dissemination or supporting microbial survival in the subject.

43. The method of claim 41 or 42, wherein the subject's response to the microbe or the microbial toxin results in thrombocytopenia, thrombosis, or enhancement of biofilm formation in the subject.

44. The method of claim 43, wherein the thrombocytopenia is caused by induction of activation and phagocytosis (*e.g.*, Survival of platelets is shortened after activation by contact with pathogens; for example, in adenovirus infection, by HIV Tat protein, fungal pathogens (*Aspergillus*, *Candida* and *Mucormycetes*) and secreted compounds of *Aspergillus*; STEC enhances platelet phagocytosis by macrophages by inducing down modulation of platelet CD47), induction of apoptosis and cell lysis (*e.g.*, *Staphylococcus aureus* and *Escherichia coli*, as well as their secreted toxins, trigger degradation of the anti-apoptotic protein Bcl-x $_L$ in platelets; Bacterial cell wall peptidoglycan stimulates platelet apoptosis; the toxins streptolysin O of *Streptococcus pyogenes* and pneumolysin of *Streptococcus pneumoniae* form pores in the platelet membrane; Dengue virus infection induces platelet apoptosis), induction of antiplatelet autoimmune antibodies via molecular mimicry (*e.g.*, Antibodies against microbial antigens of HIV, HCV, Dengue virus and *Helicobacter pylori* cross-react with platelet glycoproteins), affecting

thrombopoiesis in the bone marrow (*e.g.*, HIV proteins interact with the cell surface of platelet progenitor cells, thus inducing functional defects; Mimicry of viral proteins induces autoimmune antibodies that inhibit megakaryocyte differentiation; Megakaryocytes are infected by HIV, HCV, CMV and HHV-6; HIV modifies the cytokine pattern in the bone marrow that is necessary for thrombopoiesis; Production of thrombopoietin, a growth factor for megakaryocyte differentiation, is impaired in HCV-induced liver disease), or sequestration of platelets in the enlarged spleen (*e.g.*, HCV-induced portal hypertension results in platelet sequestration in the spleen).

45. The method of any of the claims 1-44, wherein the microbe or the microbial toxin binds to a platelet in the subject directly.

46. The method of any of the claims 1-44, wherein the microbe or the microbial toxin binds to a platelet in the subject via a bridging moiety.

47. The method of claim 46, wherein the bridging moiety is an antibody against the microbe or the microbial toxin.

48. The method of any of the claims 1-45, wherein the microbe or the microbial toxin binds to a platelet in the subject via a receptor on the platelet selected from the group consisting of GP1ba (*e.g.*, Direct binding of bacterial surface proteins or via vWF as bridging molecules), GPIa–IIa ($\alpha_2\beta_1$ integrin) (*e.g.*, Binding of rotavirus VP4 surface protein), GPIIb–IIIa ($\alpha_{2b}\beta_3$ integrin) (*e.g.*, Direct binding of bacterial surface proteins or via fibronectin and fibrinogen as bridging molecules; binding to adenovirus and hantavirus), GPVI (*e.g.*, Binding of HCV), a toll-like receptor (TLR) (*e.g.*, Direct binding of bacterial LPS), FcγRIIa (CD32) (*e.g.*, Binding of IgG in immune complexes with all pathogens), a complement receptor (*e.g.*, Binding of complement factors bound on pathogen surface; Direct binding of EBV by CR2; Binding of anaphylatoxins by C3aR and C5aR), a thrombin receptor (*e.g.*, Bacteria-induced TF catalyzes thrombin generation that activates platelets), a cytokine/chemokine receptor (*e.g.*, Sensing of inflammatory cytokines and

chemokines; Binding to HIV), a *N* -formyl peptide receptor (*e.g.*, Binding of bacteria-derived formyl peptides with subsequent gradient-driven chemotaxis), a C-type lectin (*e.g.*, Binding of HIV to DC-SIGN and CLEC-2) and a coxsackievirus and adenovirus receptor (CAR) (*e.g.*, Binding of adenovirus).

49. The method of any of the claims 1-48, wherein the microbe is a bacterium, a virus, a fungus and/or a parasite.

50. The method of claim 49, wherein the microbe is a bacterium, *e.g.*, a Gram-positive bacterium or a Gram-negative bacterium.

51. The method of claim 50, wherein the bacterium is in a genus of *Staphylococcus*, *Escherichia*, *Streptococcus* or *Helicobacter*.

52. The method of claim 51, wherein the bacterium is in the genus of *Staphylococcus*.

53. The method of claim 52, wherein the bacterium is in *S. aureus* group (*e.g.*, *S. aureus* or *S. simiae*), *S. auricularis* group (*e.g.*, *S. auricularis*), *S. carnosus* group (*e.g.*, *S. carnosus*, *S. condimenti*, *S. massiliensis*, *S. piscifermentans*, or *S. simulans*), *S. epidermidis* group (*e.g.*, *S. capitis*, *S. caprae*, *S. epidermidis*, or *S. saccharolyticus*), *S. haemolyticus* group (*e.g.*, *S. devriesei*, *S. haemolyticus*, or *S. hominis*), *S. hyicus-intermedius* group (*e.g.*, *S. chromogenes*, *S. felis*, *S. delphini*, *S. hyicus*, *S. intermedius*, *S. lutrae*, *S. microti*, *S. muscae*, *S. pseudintermedius*, *S. rostri*, or *S. schleiferi*), *S. lugdunensis* group (*e.g.*, *S. lugdunensis*), *S. saprophyticus* group (*e.g.*, *S. arlettae*, *S. cohnii*, *S. equorum*, *S. gallinarum*, *S. kloosii*, *S. leei*, *S. nepalensis*, *S. saprophyticus*, *S. succinus*, or *S. xylosus*), *S. sciuri* group (*e.g.*, *S. fleurettii*, *S. lentus*, *S. sciuri*, *S. stepanovicii*, or *S. vitulinus*), *S. simulans* group (*e.g.*, *S. simulans*) or *S. warneri* group (*e.g.*, *S. pasteuri*, or *S. warneri*).

54. The method of claim 51, wherein the bacterium is in the genus of *Escherichia*, *e.g.*, *Escherichia coli*.

55. The method of claim 51, wherein the bacterium is in the genus of *Streptococcus*.

56. The method of claim 55, wherein the bacterium is *Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus equisimilis, Streptococcus bovis, Streptococcus anginosus, Streptococcus sanguinis, Streptococcus suis, Streptococcus mitis, Streptococcus mutans,* or *Streptococcus pneumoniae.*

57. The method of claim 51, wherein the bacterium is in the genus of *Helicobacter*.

58. The method of claim 57, wherein the bacterium is *Helicobacter pylori* or a non-*pylori Helicobacter* species.

59. The method of claim 58, wherein the non-*pylori Helicobacter* species is *H. suis*, *H. baculiformis*, *H. equorum*, *H. hepaticus, H. mustelae*, *H. bilis, H. felis, H. bizzozeronii*, *H. salomonis, H. ganmani*, *H. pullorum, H. anseris*, *H. brantae, H. cinaedi* or *H. canis*.

60. The method of any of the claims 50-59, wherein the nanoparticle comprises an agent for preventing the bacterial infection, treating the bacterial infection, diagnosing the bacterial infection, prognosing the bacterial infection and/or monitoring prevention or treatment of the bacterial infection.

61. The method of claim 60, wherein the agent is an antibiotic.

62. The method of claim 61, wherein the antibiotic has a bactericidal activity or bacteriostatic activity.

63. The method of claim 61 or 62, wherein the antibiotic is a narrow-spectrum or a broad-spectrum antibiotic.

64. The method of any of the claims 61-63, wherein the antibiotic targets the bacterial cell wall (*e.g.*, penicillins and cephalosporins), targets the cell membrane (*e.g.*, polymyxins), interferes with essential bacterial enzymes (*e.g.*, rifamycins, lipiarmycins, quinolones and sulfonamides), targets protein synthesis (*e.g.*, macrolides, lincosamides and tetracyclines), or is a cyclic lipopeptide (such as daptomycin), a glycylcycline (such as tigecycline), an oxazolidinone (such as linezolid), or a lipiarmycin (such as fidaxomicin).

65. The method of any of the claims 50-64, wherein the nanoparticle comprises an agent for decreasing or neutralizing the effect of a bacterial toxin.

66. The method of claim 49, wherein the microbe is a virus.

67. The method of claim 66, wherein the virus is a dsDNA virus (*e.g.*, an adenoviruse, a herpesvirus, a poxvirus), a ssDNA virus (+ strand or "sense") DNA (*e.g.*, a parvovirus), a dsRNA virus (*e.g.*, a reovirus), (+)ssRNA virus (+ strand or sense) RNA (*e.g.*, a picornavirus, a togavirus) or a (-)ssRNA virus (– strand or antisense) RNA (*e.g.*, an orthomyxovirus, a rhabdovirus), a ssRNA-RT virus (+ strand or sense) RNA with DNA intermediate in life-cycle (*e.g.*, a retrovirus) or a dsDNA-RT virus (*e.g.*, a hepadnavirus).

68. The method of claim 66 or 67, wherein the virus is an adenovirus (*e.g.*, adenovirus serotypes 1, 2, 4, 5, 6, 7, 8, 14, 19, 36, 37, 40 and 41), a coxsackievirus, a cytomegalovirus (CMV) (*e.g.*, human CMV or human herpesvirus-5 (HHV-5)), a Dengue virus, a hantavirus (*e.g.*, a strain that causes hantavirus hemorrhagic fever with renal syndrome (HFRS) or Hantavirus pulmonary syndrome (HPS)), a hepatitis C virus (HCV), a hepatitis B virus (HBV), a herpesviridae (*e.g.*, Epstein–Barr virus (EBV), also called human herpesvirus 4 (HHV-4)), a lentivirus (*e.g.*, the human immunodeficiency virus (HIV)), a muromegalovirus (*e.g.*, human herpesvirus 6 (HHV-6)), a Roseolovirus (*e.g.*, HHV-6 and human herpesvirus 7 (HHV-7)), or a rotavirus (A, B, C, D, and E rotavirus A).

69. The method of any of the claims 66-68, wherein the nanoparticle comprises an agent, *e.g.*, an anti-viral agent, for preventing the viral infection, treating the viral infection, diagnosing the viral infection, prognosing the viral infection and/or monitoring prevention or treatment of the viral infection.

70. The method of any of the claims 66-69, wherein the anti-viral agent interferes with the ability of a virus to infiltrate a target cell (*e.g.*, an entry inhibitor and an uncoating inhibitor), targets the processes that synthesize virus components after a virus invades a cell (*e.g.*, an inhibitor or viral reverse transcription, integrase, transcription, translation, protein processing and targeting, or a protease inhibitor), targets viral assembly or targets viral release phase.

71. The method of claim 49, wherein the microbe is a fungus.

72. The method of claim 49, wherein the fungus in a genus of *Candida* (*e.g.*, *Candida albicans*), *Aspergillus* (*e.g.*, *Aspergillus fumigatus* and *Aspergillus flavus*), *Cryptococcus* (*e.g.*, *Cryptococcus neoformans* and *Cryptococcus gattii*), *Histoplasma* (*e.g.*, *Histoplasma capsulatum*), *Mucormycetes* (*e.g.*, *Rhizopus oryzae* (or *Rhizopus microsporus*), *Mucor* species, *Lichtheimia corymbifera*, and *Rhizomucor pusillus*), *Pneumocystis* (*e.g.*, *Pneumocystis jirovecii*), or *Stachybotrys* (*e.g.*, *Stachybotrys chartarum*).

73. The method of claim 71 or 72, wherein the nanoparticle comprises an agent, *e.g.*, an anti-fungal agent, for preventing the fungal infection, treating the fungal infection, diagnosing the fungal infection, prognosing the fungal infection and/or monitoring prevention or treatment of the fungal infection.

74. The method of any of the claims 71-73, wherein the anti-fungal agent is a polyene antifungal agent, an imidazole antifungal agent, a triazole antifungal agent, a thiazole antifungal agent, an allylamine or an echinocandin.

75. The method of claim 49, wherein the microbe is a parasite.

76. The method of claim 75, wherein the parasite in a genus of *Plasmodium* (*e.g.*, *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesii*), *Schistosoma* (*Schistosoma guineensis*, *Schistosoma intercalatum*, *Schistosoma haematobium*, *Schistosoma japonicum*, *Schistosoma malayensis*, *Schistosoma mansoni* and *Schistosoma mekongi*) or *Toxoplasma gondii*.

77. The method of claim 75 or 76, wherein the nanoparticle comprises an agent, *e.g.*, an anti- parasitic agent, for preventing the parasitic infection, treating the parasitic infection, diagnosing the parasitic infection, prognosing the parasitic infection and/or monitoring prevention or treatment of the parasitic infection.

78. The method of any of the claims 75-77, wherein the anti-parasitic agent is an anti-worm agent, an anti-malaria agent or an anti-*Toxoplasma gondii* agent.

79. The method of claim 78, wherein the an anti-malaria agent is used for treating malaria in a subject with suspected or confirmed infection, for preventing infection in a subject, or for routine intermittent treatment of certain groups of subjects in an endemic region.

80. The method of claim 78 or 79, wherein the anti-malaria agent is a quinine and related agent, chloroquine, amodiaquine, pyrimethamine, proguanil, a sulfonamide, mefloquine, atovaquone, primaquine, artemisinin and derivative, halofantrine, doxycycline, or clindamycin.

81. The method of any of the claims 1-80, which further comprises administering another active ingredient to the subject.

82. The method of claim 81, wherein the other active ingredient is used for preventing and/or treating infection by a platelet-targeting microbe in a subject.

83. The method of claim 81 or 82, wherein the other active ingredient comprises a red blood cell membrane coated nanoparticle comprising an inner core comprising a non-cellular material and an outer surface comprising a cellular membrane derived from a red blood cell.

84. The method of claim 83, wherein the red blood cell membrane coated nanoparticle to decrease or neutralize the effect of a RBC-targeting toxin produced by the microbe in a subject.

85. The method of claim 83 or 84, wherein the red blood cell membrane coated nanoparticle further comprises active ingredient for preventing and/or treating infection by a microbe in a subject.

86. The method of claim 85, wherein the red blood cell membrane coated nanoparticle comprises active ingredient for preventing and/or treating infection by a RBC-targeting microbe in a subject.

87. The method of any of the claims 1-86, which further comprises administering a pharmaceutically acceptable carrier or excipient to the subject.

88. The method of any of the claims 1-87, wherein the nanoparticle is administered via a medicament delivery system.

89. The method of any of the claims 1-88, which further comprises assessing efficacy of the nanoparticle and/or the another active ingredient in preventing and/or treating infection by a platelet-targeting microbe in a subject.

90. The method of any of the claims 1-89, wherein the nanoparticle, alone or in combination with other active ingredient(s), is administered via oral, parenteral, rectal, nasal, topical, or ocular routes, or by inhalation.

91. The method of claim 90, wherein the parenteral administration is via intravenous, intramuscular, intraperitoneal, intranasal, or subcutaneous route.

92. Use of an effective amount of a nanoparticle for the manufacture of a medicament for preventing and/or treating infection by a platelet-targeting microbe in a subject, wherein said nanoparticle comprises:

a) an inner core comprising a non-cellular material,

b) an outer surface comprising a cellular membrane derived from a platelet; and optionally

c) an agent for preventing said infection, treating said infection, diagnosing said infection, prognosing said infection and/or monitoring prevention or treatment of said infection.

93. A combination for preventing and/or treating infection by a platelet-targeting microbe in a subject, which combination comprises an effective amount of a nanoparticle and an effective amount of a second prophylactic or therapeutic agent for preventing and/or treating infection by a platelet-targeting microbe in a subject, wherein said nanoparticle comprises:

a) an inner core comprising a non-cellular material,

b) an outer surface comprising a cellular membrane derived from a platelet; and optionally

c) an agent for preventing said infection, treating said infection, diagnosing said infection, prognosing said infection and/or monitoring prevention or treatment of said infection.

94. A pharmaceutical composition comprising a combination of claim 93 admixed with at least one pharmaceutically acceptable carrier or excipient.

95. A method for preventing and/or treating infection by a platelet-targeting microbe in a subject, which method comprises administering, to a subject in need, or to cells of said subject, an effective amount of a combination of claim 93, or a pharmaceutical composition of claim 94.

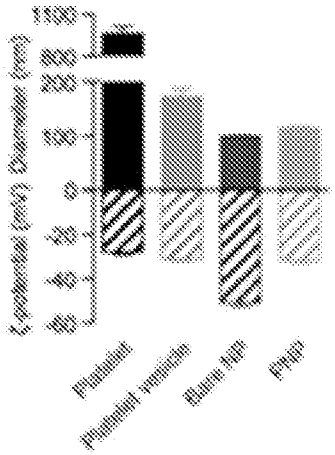


Figure 1A

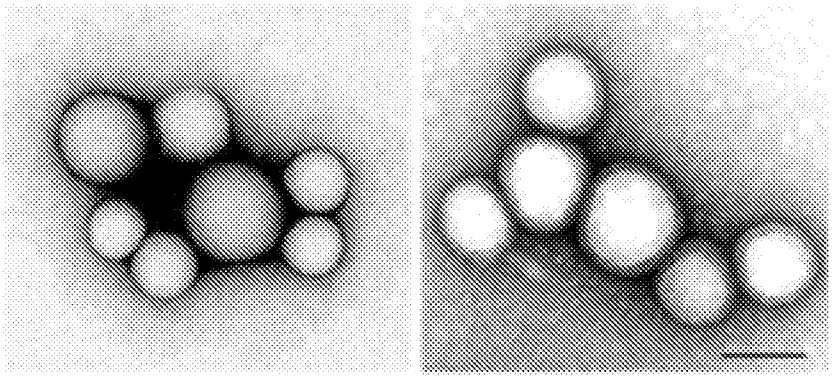


Figure 1B

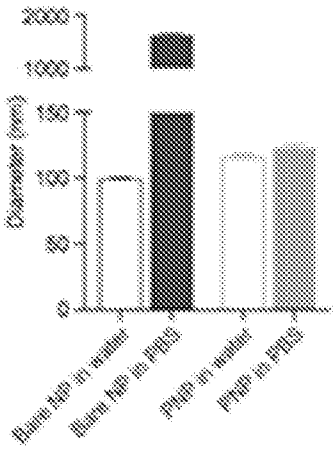


Figure 1C

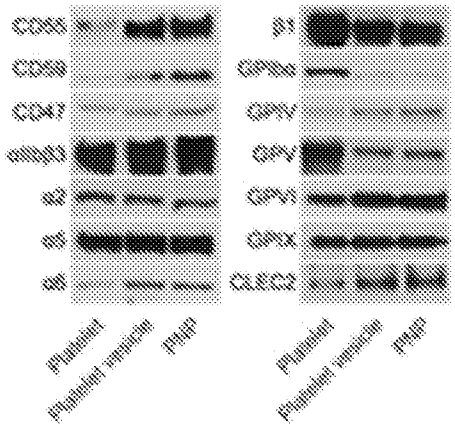


Figure 1D

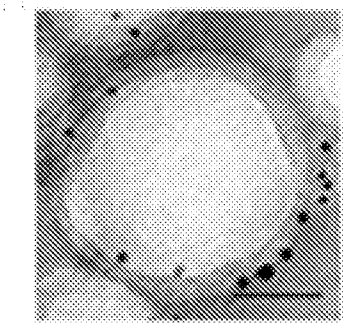


Figure 1E

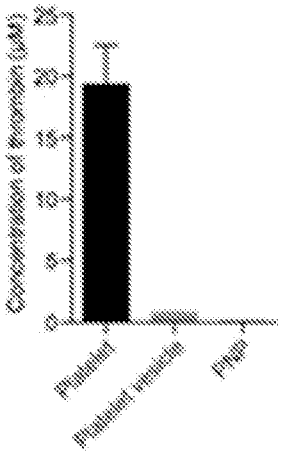


Figure 1F

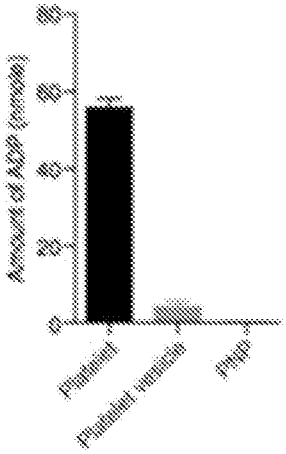


Figure 1G

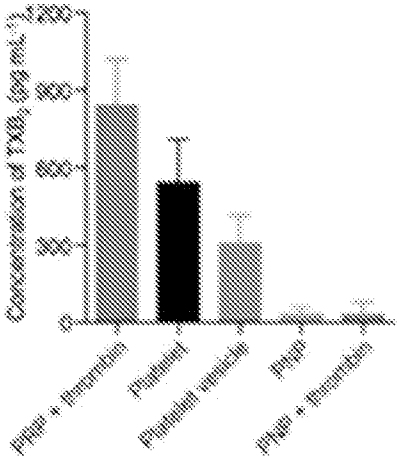


Figure 1G

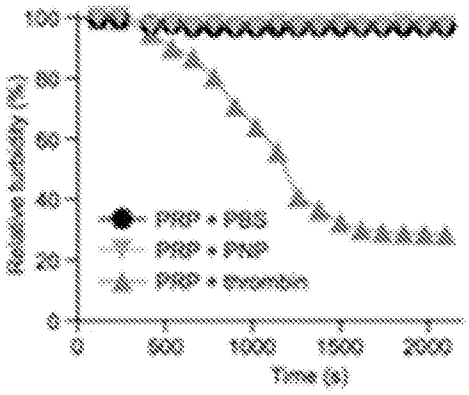


Figure 1H

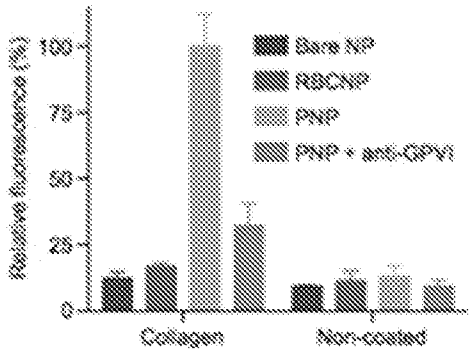


Figure 2A

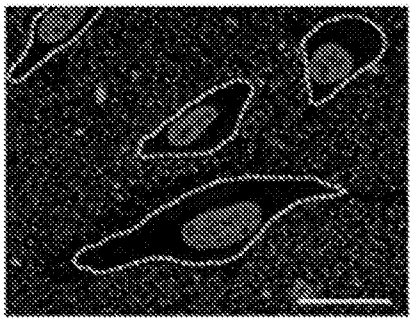


Figure 2B

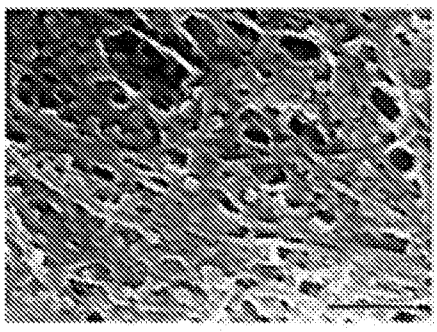


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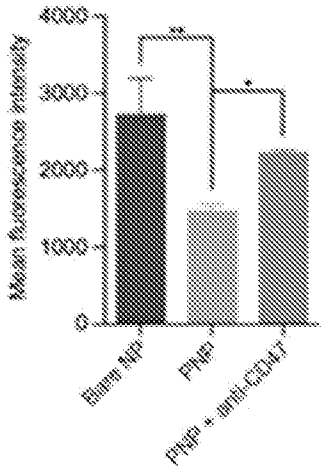


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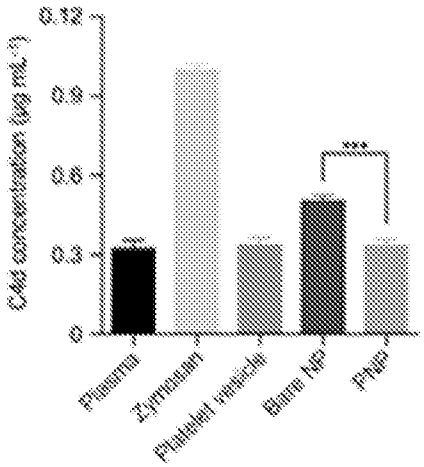


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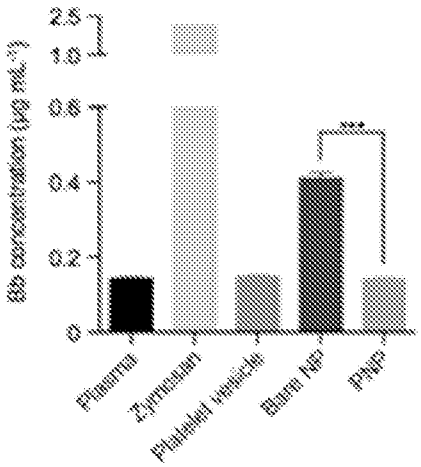


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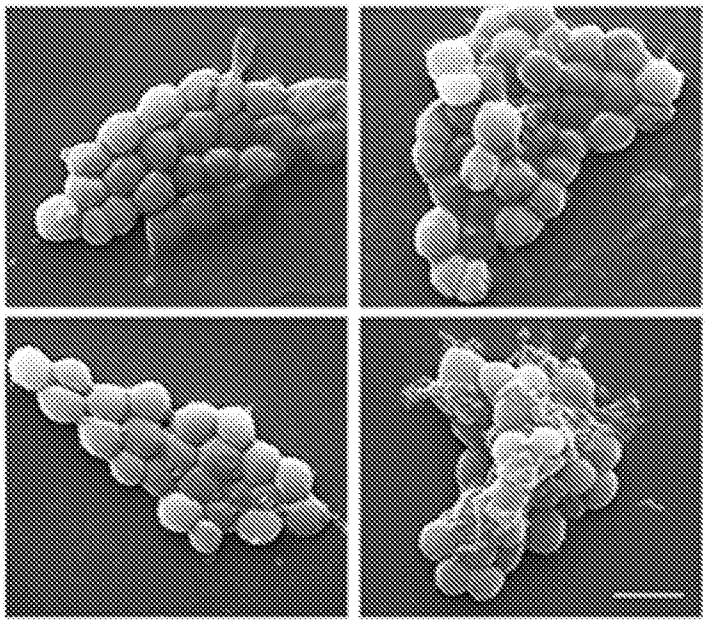


Figure 3A

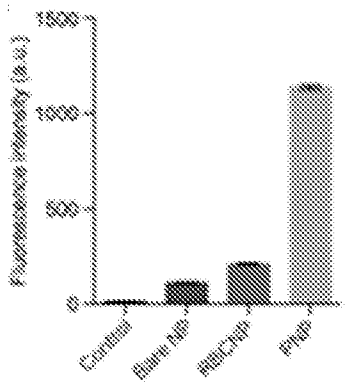


Figure 3B

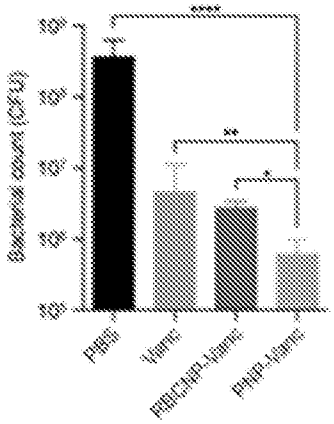


Figure 3C

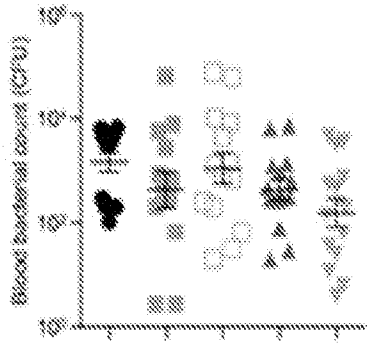


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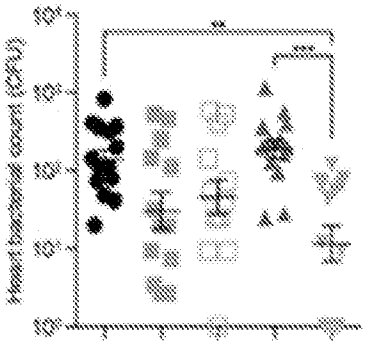


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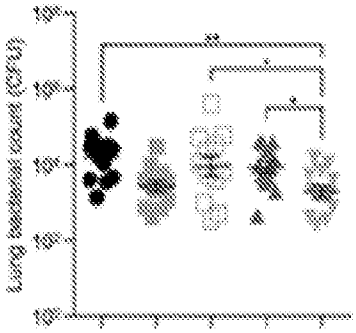


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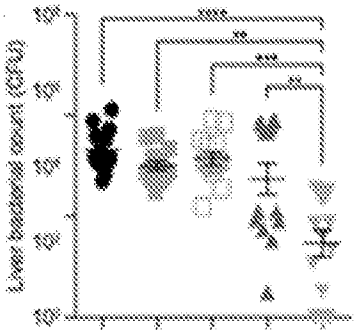


Figure 3G

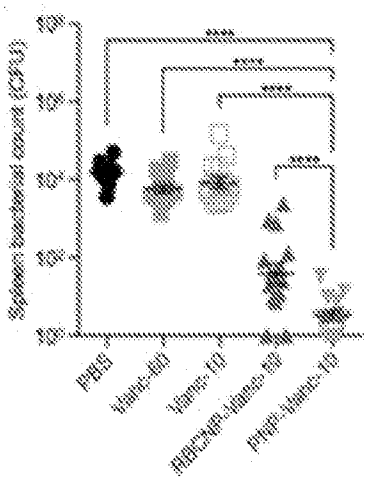


Figure 3H

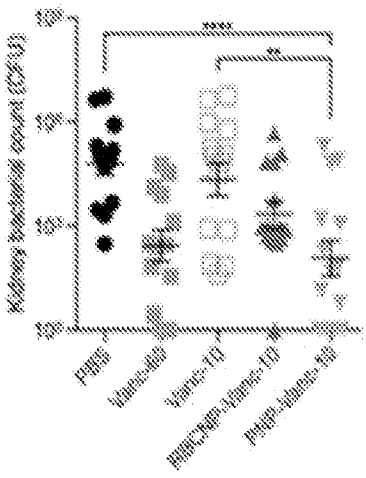


Figure 3I

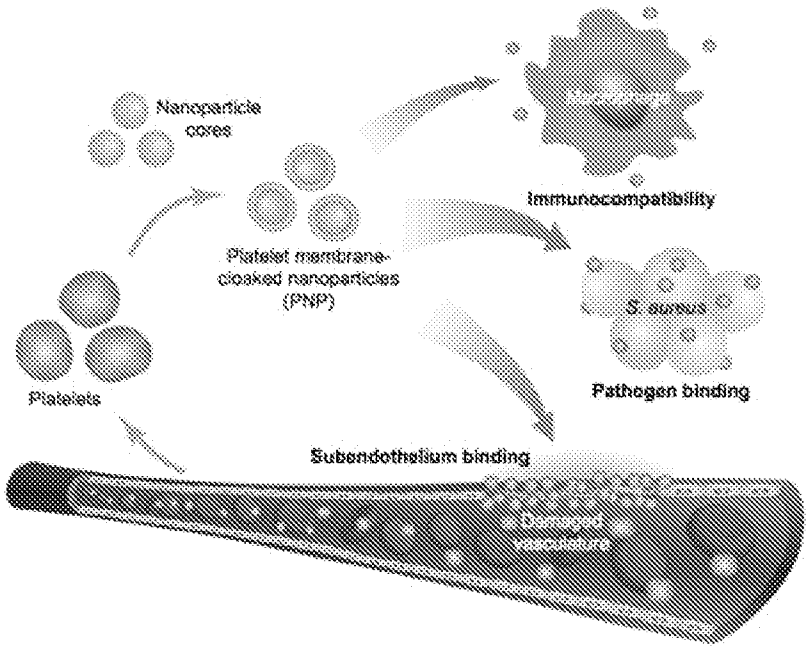


Figure 4A

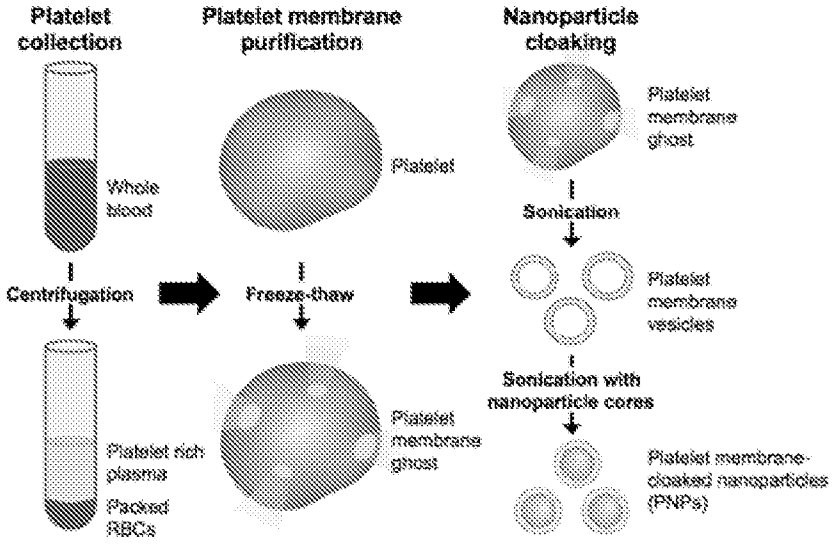


Figure 4B

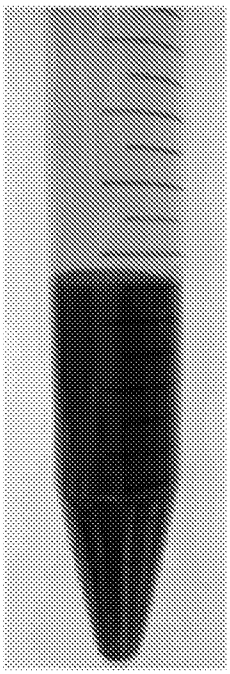


Figure 5A

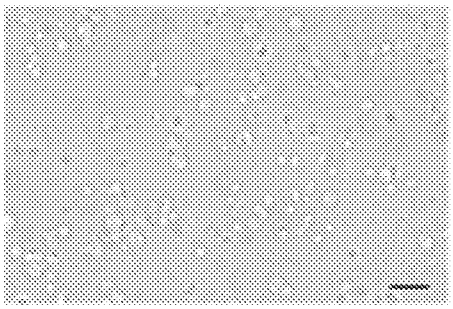


Figure 5B

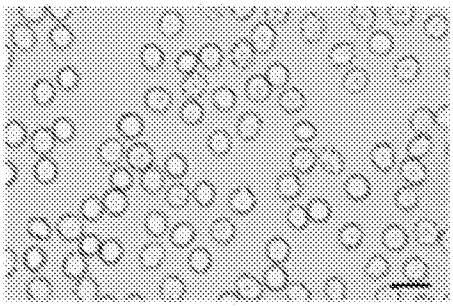


Figure 5C

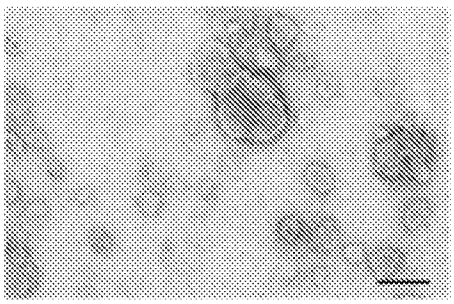


Figure 5D

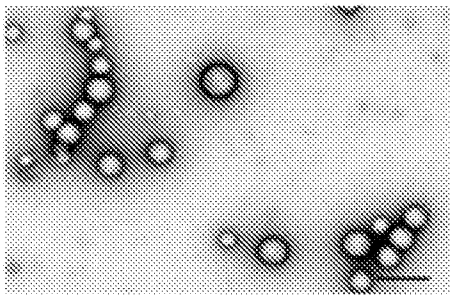


Figure 5E

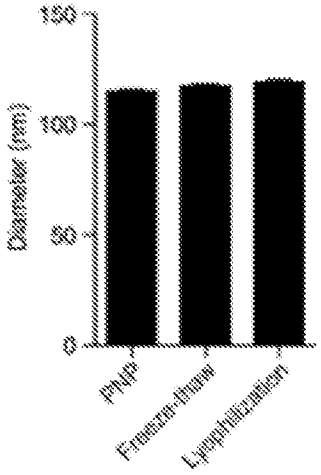


Figure 5F

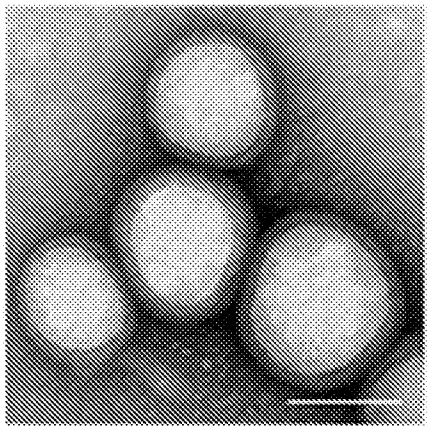


Figure 5G

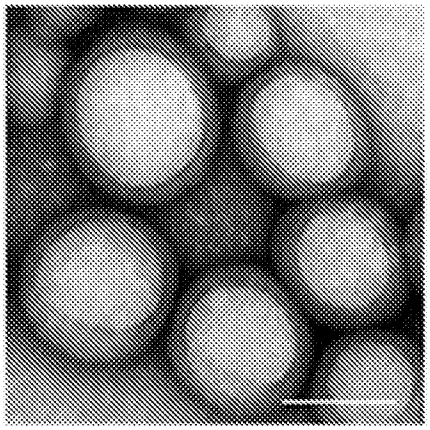


Figure 5H

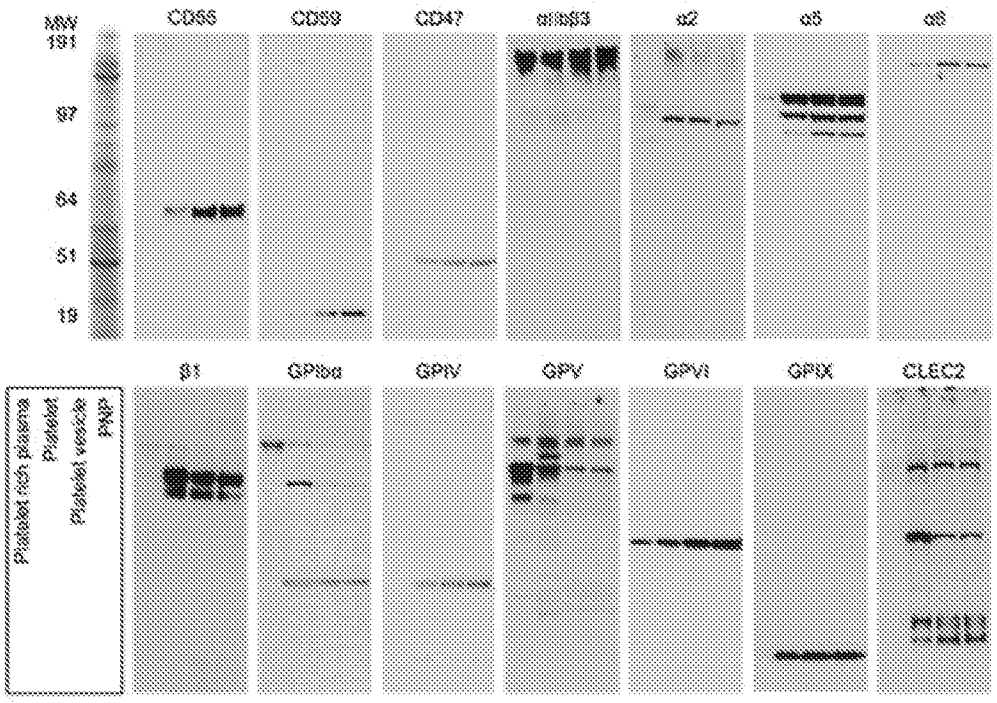

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51
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CD59
CD47
αIIbβ3
α2
α5
α6
β1
GPIbα
GPIV
GPV
GPVI
GPIX
CLEC2
Platelet rich plasma
Platelet
Platelet wash
Platelet wash resting
PNK

Figure 6

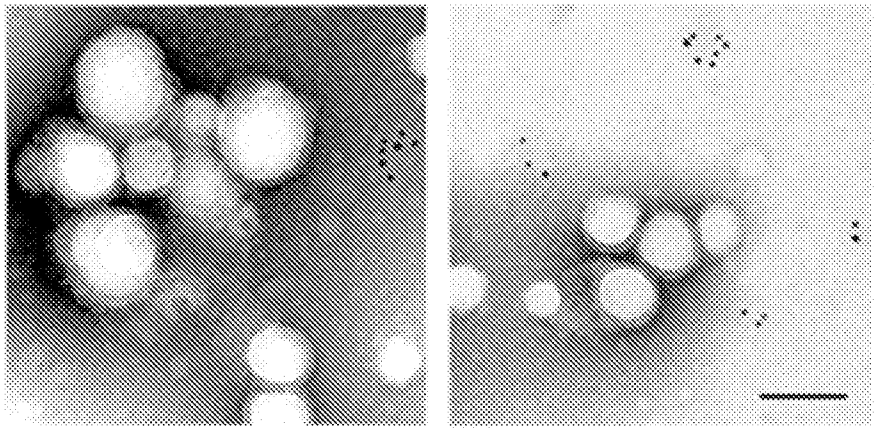


Figure 7A

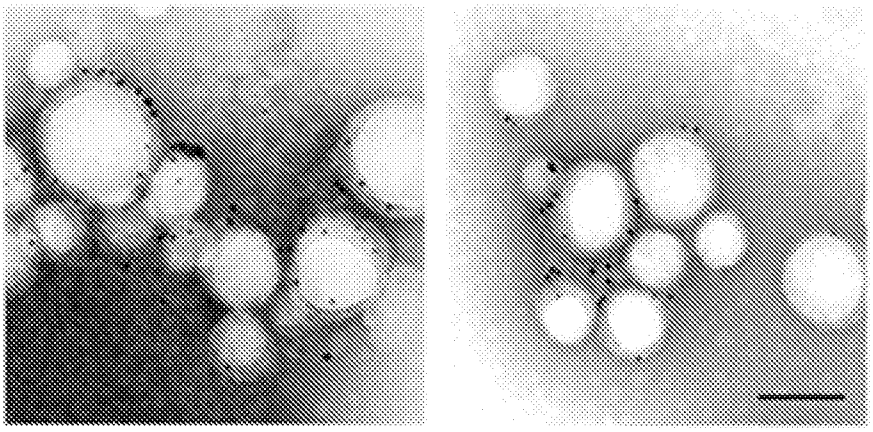


Figure 7B

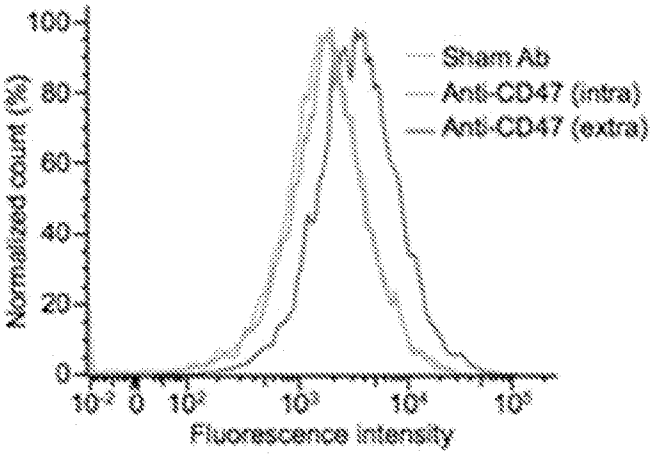


Figure 7C

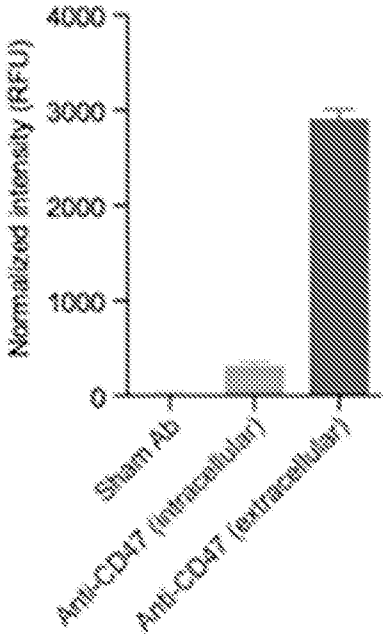


Figure 7D

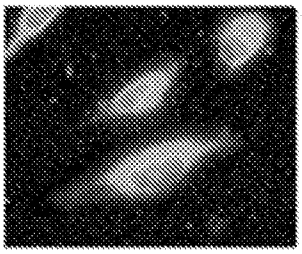


Figure 8A

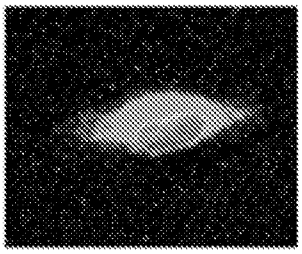


Figure 8B

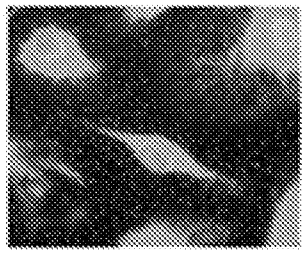


Figure 8C

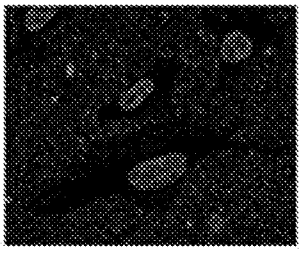


Figure 8D

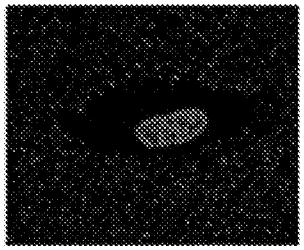


Figure 8E

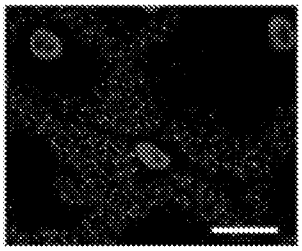


Figure 8F

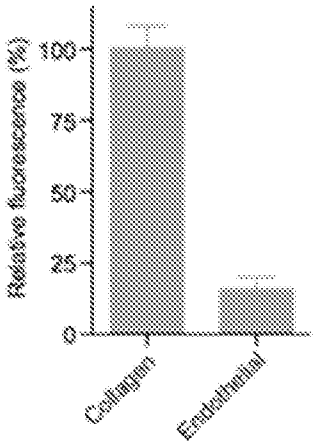


Figure 8G

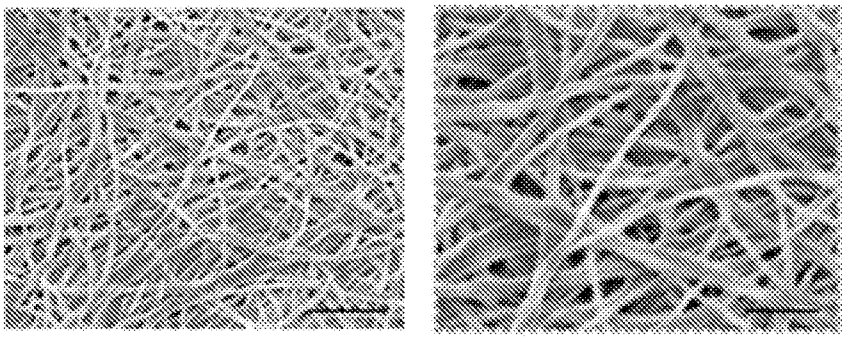


Figure 8H

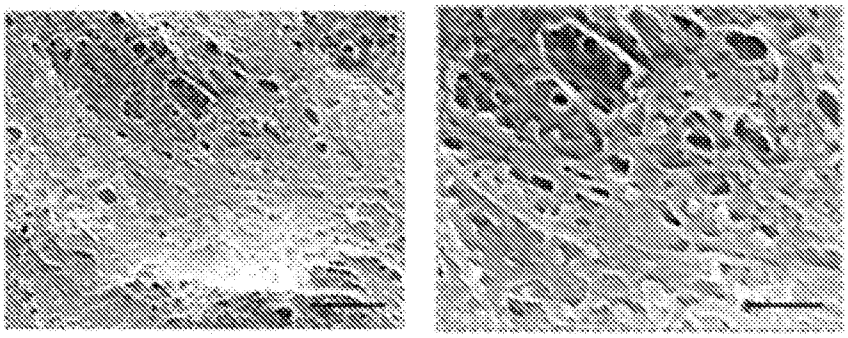


Figure 8I

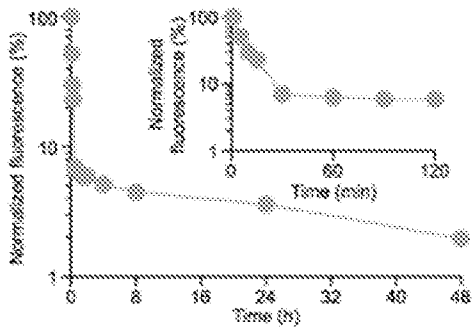


Figure 9A

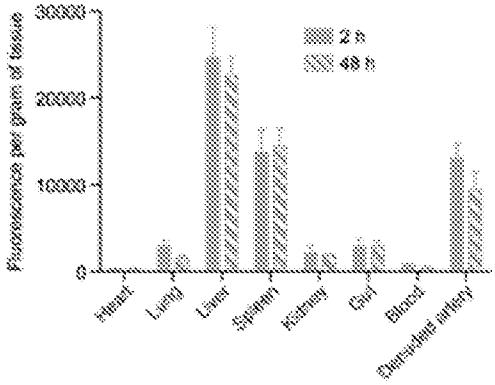


Figure 9B

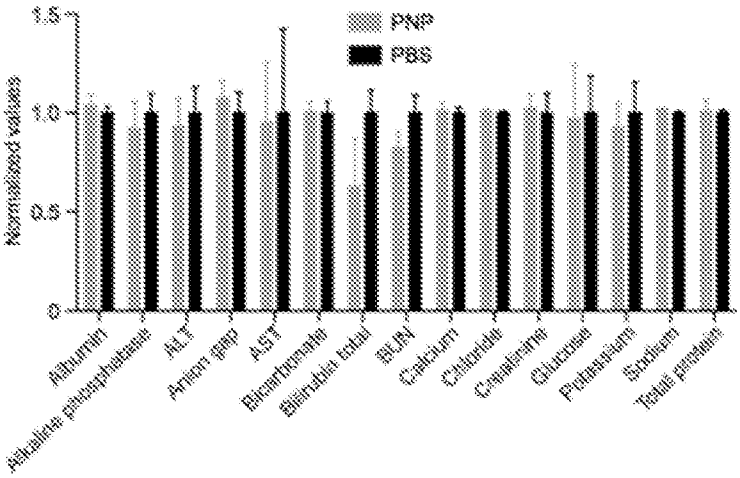


Figure 9C

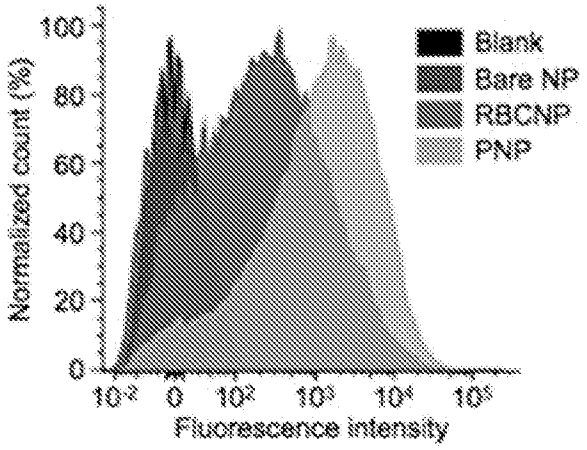


Figure 10A

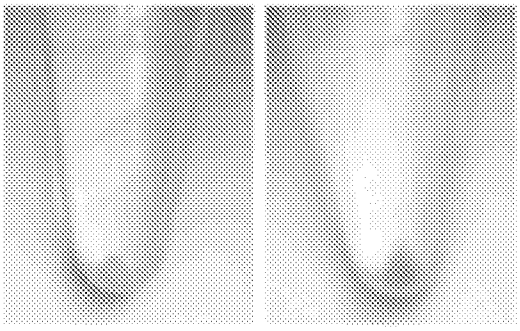


Figure 10B

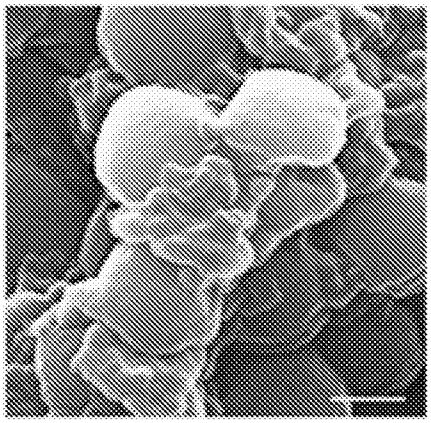


Figure 10C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/36132

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-30, 32-91
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/36132

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 9/14, 45/06, 45/08, 47/00 (2016.01)
CPC - B82Y 5/00; A61K 4506, 9/148, 39/085, 2039/55555
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: A61K 9/14, 45/06, 45/08, 47/00 (2016.01)
CPC: B82Y 5/00; A61K 4506, 9/148, 39/085, 2039/55555

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC: B82Y 5/00; A61K 4506, 9/148, 39/085, 2039/55555 (text search)
USPC: 977/703, 773; 424/489, 532, 237.1

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic data bases: PatBase, Google Patents; Google Scholar
Search terms: Nanoparticle, inner core, outer core, drug delivery, platelet, platelet-targeting microbe (e.g., bacterium, virus, fungus), infection, pharmaceutical composition, prevention, treatment

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2013/0337066 A1 (The Regents of the University of California) 19 December 2013 (19.12.2013). Especially para [0007], [0009], [0010], [0015], [0018], [0020], [0117], [0118]	1-6, 31, 92-95
Y	YEAMAN. Platelets in defense against bacterial pathogens. Cell Mol Life Sci February 2010 Vol 67 No 4 pages 525-544. Especially pg 529 col 2 para 3.	1-6, 31, 92-95

☐ Further documents are listed in the continuation of Box C. ☐

* 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
15 August 2016 (15.08.2016)	08 SEP 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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