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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 October 2016 (27.10.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/172069 A1

(51) International Patent Classification:
A61K 9/14 (2006.01) *A61K 31/337* (2006.01)
A61K 47/42 (2006.01) *A61K 9/107* (2006.01)

(21) International Application Number:
PCT/US2016/028221

(22) International Filing Date:
19 April 2016 (19.04.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/150,201 20 April 2015 (20.04.2015) US

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

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

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

[Continued on next page]

(54) Title: METHODS OF TREATING INFLAMMATION ASSOCIATED AIRWAY DISEASES AND VIRAL INFECTIONS

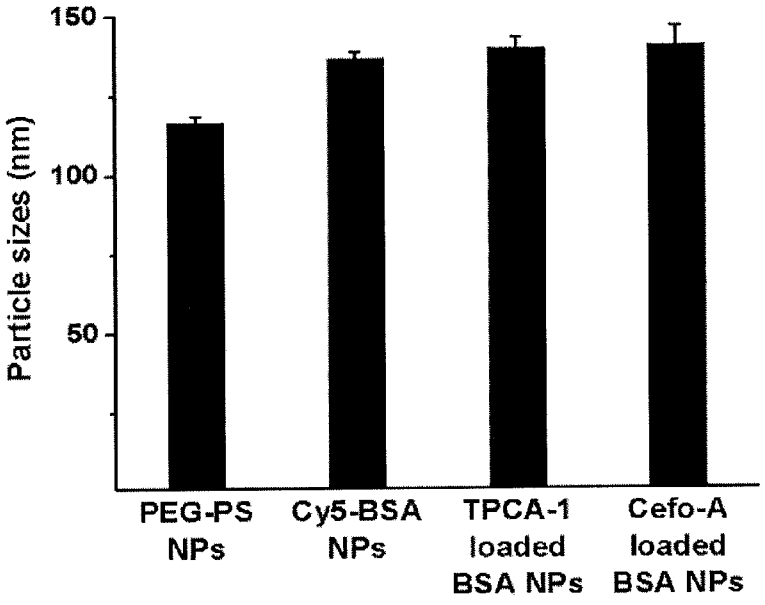

FIG. 1A

(57) Abstract: The present disclosure provides nanoparticle compositions comprising a drug and an albumin protein for the treatment of diseases with an inflammatory component. Methods of treatment and methods of transporting a nanoparticle composition across a barrier of a blood vessel are also provided.

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— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

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

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

METHODS OF TREATING INFLAMMATION ASSOCIATED AIRWAY DISEASES AND VIRAL INFECTIONS

[001] This application claims the benefit under 35 USC § 119(e) of U.S. Provisional Application Serial No. 62/150,201, filed on April 20, 2015, the entire disclosure of which is incorporated herein by reference.

GOVERNMENT RIGHTS

[002] This invention was made with government support under Grant No. K25HL111157, awarded by NIH. The government has certain rights in the invention.

TECHNICAL FIELD

[003] The present disclosure provides nanoparticle compositions comprising a drug and an albumin protein for the treatment of diseases with an inflammatory component. Methods of treatment and methods of transporting a nanoparticle composition across a barrier of a blood vessel are also provided.

BACKGROUND AND SUMMARY OF THE INVENTION

[004] Nanomedicine to treat extravascular diseases is premised on the ability to achieve targeted drug delivery and desirable drug retention in a specific location. For medical benefits, nanoparticles (NPs) formulated to be therapeutic should be able to cross a blood vessel barrier in a patient. However, current approaches are insufficient for actively delivering NPs across this barrier. In blood vessels, the endothelium forms a monolayer lining the vessel wall to regulate plasma permeability into tissues. Generally, the physiological barrier of an inter-endothelial passage is smaller than 3 nm. Therefore, any particles larger than this size have difficultly to penetrate the endothelial gap into extravascular tissues. As a result, the EPR (enhanced permeation and retention) effect is often used for cancer targeting of drugs because leaky vessels could assist in the tissue deposition of therapeutic NPs. For example, in cancer therapies, tumor vasculatures are more permeable than normal tissues, which facilitates drug-loaded NPs to efficiently accumulate into tumor vasculature, therefore increasing therapeutic efficacy compared with free drugs.

[005] However, EPR is a passive process and particle-size dependent, and effects are very difficult to achieve. This passive drug delivery is strongly dependent on the size of nanoparticle carriers, and their circulation time in the bloodstream. Thus, there exists a need to develop

more effective routes for active delivery of NPs across a blood vessel barrier to achieve targeted drug delivery and preferable drug retention.

[006] In the body, inflammation is a result of the innate and adaptive immune responses characterized by a marked increase of immune cells and their trafficking from bloodstream to pathogenic tissues. Millions of polymorphonuclear neutrophils (a type of white blood cells) can rapidly respond to inflammation through neutrophil activation, adhesion to and migration across endothelial vessels into inflammatory tissues *via* the intercellular route. Therefore, neutrophils could be an excellent carrier to mediate the delivery of therapeutic NPs across the endothelial vessel barrier and to specifically target diseased tissues.

[007] Nanotechnology has demonstrated to be a powerful tool to design nanotherapeutics which can incorporate therapeutic agents inside NPs and target desired cell types or organs by biologically functioning nanoparticle surfaces. Denatured albumin NPs can specifically target activated neutrophils adherent to the vessel wall using intravital microscopy of live mouse cremaster venules. The instant disclosure investigates whether these activated neutrophils can be utilized as a carrier to deliver nanoparticle cargo across the blood vessel barrier.

[008] The present disclosure provides that therapeutic NPs can be delivered across the endothelial vessel wall using the neutrophil transmigration pathway. Advantageously, parenterally administered albumin NPs can be specifically internalized by activated neutrophils. Subsequently, the neutrophils containing NPs can cross the blood vessel wall because neutrophils are able to transmigrate in response to inflammation induced by the pathogen invasion. According to the methods of the present disclosure, a range of therapeutics can be delivered across the blood vessel barrier, thus improving therapies of various diseases related to inflammation. For example, diseases related to inflammation include but are not limited to acute inflammatory diseases, chronic inflammatory diseases, infections, and cancer.

[009] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

[0010] The following numbered embodiments are contemplated and are non-limiting:

1. A nanoparticle composition comprising a drug and an albumin protein.

2. The nanoparticle composition of clause 1 wherein the drug is an antibiotic.

3. The nanoparticle composition of clause 1 wherein the drug is an anti-inflammatory drug.

4. The nanoparticle composition of clause 1 wherein the drug is an anticancer drug.

5. The nanoparticle composition of clause 1 wherein the drug is an NF-κB inhibitor.

6. The nanoparticle composition of clause 5 wherein the NF-κB inhibitor is TPCA-1.

7. The nanoparticle composition of clause 1 wherein the drug is cefoperazone acid.

8. The nanoparticle composition of any one of clauses 1 to 7 wherein the albumin protein is a bovine serum albumin (BSA) protein.

9. The nanoparticle composition of clause 8 wherein the BSA protein is a denatured BSA protein.

10. The nanoparticle composition of any one of clauses 1 to 9 wherein the nanoparticle composition is conjugated to a polyethylene glycol moiety.

11. The nanoparticle composition of any one of clauses 1 to 10 wherein the nanoparticle composition has a diameter between about 100 nm to about 150 nm.

12. A method of transporting a nanoparticle composition across a blood vessel, said method comprising the step of administering the nanoparticle composition to a patient comprising the blood vessel, wherein the nanoparticle composition is transported by one or more neutrophils across a barrier of the blood vessel.

13. The method of clause 12 wherein the transporting across the blood vessel is via a transcellular pathway.

14. The method of clause 12 wherein the transporting across the blood vessel is via a neutrophil transmigration pathway.

15. The method of any one of clauses 12 to 14 wherein the neutrophil is a polymorphonuclear neutrophil.

16. The method of any one of clauses 12 to 14 wherein the neutrophil is an activated neutrophil.

17. The method of any one of clauses 12 to 16 wherein the neutrophil comprises a Fcγ receptor.

18. The method of any one of clauses 12 to 17 wherein the nanoparticle composition is internalized into the neutrophil.

19. The method of any one of clauses 12 to 18 wherein the barrier of the blood vessel is an endothelial vessel barrier.

20. The method of any one of clauses 12 to 18 wherein the blood vessel is a lung blood vessel.

21. The method of any one of clauses 12 to 20 wherein the nanoparticle composition comprises a drug and an albumin protein.

22. The method of clause 21 wherein the drug is an antibiotic.

23. The method of clause 21 wherein the drug is an anti-inflammatory drug.

24. The method of clause 21 wherein the drug is an anticancer drug.

25. The method of clause 21 wherein the drug is an NF-κB inhibitor.

26. The method of clause 25 wherein the NF-κB inhibitor is TPCA-1.

27. The method of clause 21 wherein the drug is cefoperazone acid.

28. The method of any one of clauses 12 to 27 wherein the albumin protein is a bovine serum albumin (BSA) protein.

29. The method of clause 28 wherein the BSA protein is a denatured BSA protein.

30. The method of any one of clauses 12 to 29 wherein the nanoparticle composition is conjugated to a polyethylene glycol moiety.

31. The method of any one of clauses 12 to 30 wherein the nanoparticle composition has a diameter between about 100 nm to about 150 nm.

32. The method of any one of clauses 12 to 31 wherein the transporting across the blood vessel enhances the delivery of the drug to a desired site.

33. The method of any one of clauses 12 to 32 wherein the method further comprises administering a neutrophil migration drug to the patient.

34. The method of clause 33 wherein the neutrophil migration drug is PD98059.

35. The method of clause 33 or clause 34 wherein the neutrophil migration drug enhances transportation of neutrophils across the barrier of the blood vessel.

36. A method of treating a disease in a patient in need thereof, said method comprising the step of administering a nanoparticle composition to the patient, wherein the administration of the nanoparticle composition reduces one or more symptoms associated with the disease.

37. The method of clause 36 wherein the disease is an inflammatory disease.

38. The method of clause 37 wherein the inflammatory disease is an acute inflammatory disease.

39. The method of clause 37 wherein the inflammatory disease is a chronic inflammatory disease.

40. The method of clause 37 wherein the inflammatory disease is cancer.

41. The method of clause 37 wherein the inflammatory disease is sepsis.

42. The method of clause 37 wherein the inflammatory disease is a lung injury.

43. The method of clause 42 wherein the lung injury is an acute lung injury.

44. The method of clause 42 wherein the lung injury is a chronic lung injury.

45. The method of clause 36 wherein the disease is an infection.

46. The method of clause 45 wherein the infection is a bacterial infection.

47. The method of clause 45 wherein the infection is a viral infection.

48. The method of clause 45 wherein the infection is a fungal infection.

49. The method of any one of clauses 36 to 48 wherein the administration of the nanoparticle composition comprises transportation across a blood vessel barrier in the patient.

50. The method of clause 49 wherein the transporting across the blood vessel is via a transcellular pathway.

51. The method of clause 49 wherein the transporting across the blood vessel is via a neutrophil transmigration pathway.

52. The method of clause 51 wherein the neutrophil is a polymorphonuclear neutrophil.

53. The method of clause 51 wherein the neutrophil is an activated neutrophil.

54. The method of any one of clauses 51 to 53 wherein the neutrophil comprises a Fcγ receptor.

55. The method of any one of clauses 36 to 54 wherein the nanoparticle composition is internalized into the neutrophil.

56. The method of clause 51 wherein the barrier of the blood vessel is an endothelial vessel barrier.

57. The method of clause 51 wherein the blood vessel is a lung blood vessel.

58. The method of clause 49 wherein the transporting across the blood vessel enhances the delivery of the drug to a desired site.

59. The method of any one of clauses 36 to 58 wherein the method further comprises administering a neutrophil migration drug to the patient.

60. The method of clause 59 wherein the neutrophil migration drug is PD98059.

61. The method of clause 59 or clause 60 wherein the neutrophil migration drug enhances transportation of neutrophils across the barrier of the blood vessel.

62. The method of any one of clauses 36 to 61 wherein the administration is a parenteral administration.

63. The method of clause 62 wherein the parenteral administration is an intravenous administration.

64. The method of any one of clauses 36 to 63 wherein the drug is an antibiotic.

65. The method of any one of clauses 36 to 63 wherein the drug is an anti-inflammatory drug.

66. The method of any one of clauses 36 to 63 wherein the drug is an anticancer drug.

67. The method of any one of clauses 36 to 63 wherein the drug is an NF-κB inhibitor.

68. The method of clause 67 wherein the NF-κB inhibitor is TPCA-1.

69. The method of any one of clauses 36 to 63 wherein the drug is cefoperazone acid.

70. The method of any one of clauses 36 to 69 wherein the albumin protein is a bovine serum albumin (BSA) protein.

71. The method of clause 70 wherein the BSA protein is a denatured BSA protein.

72. The method of any one of clauses 36 to 71 wherein the nanoparticle composition is conjugated to a polyethylene glycol moiety.

73. The method of any one of clauses 36 to 72 wherein the nanoparticle composition has a diameter between about 100 nm to about 150 nm.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better

understood by reference to one or more of these drawings in combination with the detailed description of the specification embodiments presented herein.

[0012] Figures 1A-1D show particle sizes (FIG. 1A and FIG. 1B) and zeta potentials (FIG. 1C) of the NPs determined by dynamic light scattering. The particle sizes and zeta potentials of various NPs are in the range of 115 to 140 nm and -25 to -35 mV (0.2 mg/mL in 5% glucose). Particle sizes (FIG. 1D) of the Cy5-BSA NPs in 5% glucose (left bars) and PBS, pH 7.4 (right bars) upon dilution at 1/10, 1/100 and 1/1000 of the original concentration of the NPs, which was 2 mg/mL. After dilution, there were no significant changes for particle sizes of the NPs.

[0013] Figures 2A-2G show neutrophils mediate delivery of albumin nanoparticles across blood vessel barrier moving to inflammation sites. (FIG. 2A) The concept of neutrophil-mediated delivering of therapeutic albumin NPs. (FIG. 2B) Intravital microscopic images of TNF-α-induced inflammation of live mouse cremaster venules, 30 min after intravenous (i.v.) injection of Cy5-BSA NPs (80 μg/mouse) and Alexa Fluor-488-labeled anti-mouse Gr-1 (1.5μg/mouse) to mark neutrophils. Fluorescence confocal microscopy of neutrophils from bronchoalveolar lavage fluid (BALF) 2 hours (FIG. 2C) and 20 hours (FIG. 2D) after i.v. injection of Cy5-BSA NPs (neutrophils were labelled by Alexa Fluor 488-labeled anti-mouse Gr-1 antibody, green). Nucleus were stained by DAPI (blue). (FIG. 2E) Flow cytometry of BALF after i.v. injection of Cy5-BSA NPs. Neutrophils were stained as described above. (FIG. 2F) Percentage of neutrophils internalizing NPs in BALF, obtained from flow cytometry. (FIG. 2G) Percentage of neutrophils internalizing NPs in blood of mice challenged with and without LPS, obtained from flow cytometry at 2 hours (left bars), 10 hours (middle bars), and 20 hours (right bars). 16mg/kg of NPs were administered in mice 4 h after LPS challenge (8 mg/kg). All data represent means ± SD (n=3-4 mice per group).

[0014] Figures 3A-3B show flow cytometry of BALF in healthy mice (without LPS treatment), which was collected at 20 h after intravenous (i.v.) injection of (FIG. 3A) Cy5-BSA NPs or (FIG. 3B) PEG-PS NPs in healthy mice. Few neutrophils and neutrophils internalizing NPs can be observed.

[0015] Figure 4 shows flow cytometry of BALF with LPS treatment but without the injection of Cy5-BSA NPs, which was collected at 2, 10 and 20 h. The percentage of neutrophils increased with time. The neutrophils were labelled with anti-Gr-1 488 antibody.

[0016] Figures 5A-5C show flow cytometry of neutrophils in blood with (FIG. 5A) LPS treatment, (FIG. 5B) without LPS treatment after the administration of CY5-BSA NPs and

(FIG. 5C) without both LPS treatment and administration of Cy5-BSA NPs, which was collected at 2, 10 and 20 h. The percentage of neutrophils containing Cy5-BSA NPs decreased from 2 to 20 h in mice with LPS challenge, while less than 1% of neutrophils contained BSA NPs in healthy mice.

[0017] Figures 6A-6E show LPS-induced lung permeability does not contribute transport of albumin NPs across blood vessel barrier. (FIG. 6A) Concentrations of proteins in BALF after i.v. injection of 5% glucose (left bars), Cy5-BSA NPs (middle bars), or PEG-PS NPs (right bars) in mice 4 hours after LPS challenge. (FIG. 6B) Flow cytometry of neutrophils in BALF after the administration of PEG-PS NPs in mice 4 h after LPS challenge (neutrophils were labelled by Alexa Fluor 647-labeled anti-mouse Gr-1 antibody). (FIG. 6C) Percentage of neutrophils internalizing NPs in BALF, obtained from flow cytometry. Concentrations of NPs in plasma (FIG. 6D) and BALF (FIG. 6E; 2 hours at left bars; 10 hours at middle bars; 20 hours at right bars) after i.v. injection of NPs (16mg/kg). NPs were administered in mice 4 h after LPS challenge (8 mg/kg). All data represent means ± SD (n=3-4 mice per group). N.D., not detected.

[0018] Figure 7 shows flow cytometry of BALF with LPS treatment but without the injection of PEG-PS NPs, which was collected at 2, 10 and 20 h. The percentage of neutrophils increased with time. The neutrophils were labelled with anti-Gr-1 647 antibody.

[0019] Figures 8A-8B show fluorescence confocal microscopy of neutrophils in BALF 2 hours (FIG. 8A) and 20 hours (FIG. 8B) after injection of PEG-PS NPs (neutrophils were labeled by Alexa Fluor 647 anti-mouse Gr-1 antibody). Nuclei were stained by DAPI.

[0020] Figures 9A-9C shows neutrophil depletion prevents the delivery of albumin NPs across blood vessel barrier. (FIG. 9A) Concentrations of protein in BALF, (FIG. 9B) number of neutrophils in blood and BALF and (FIG. 9C) Cy5-BSA NPs in BALF with or without the i.p. injection of anti-Gr-1 antibody. Cy5-BSA NPs and Gr-1 antibody were i.v. and i.p. injected 4 h after LPS challenge, respectively. The samples were collected 20 h after the administration of NPs. All data represent means ± SD (n=3-4 mice per group). N.D., not detected.

[0021] Figures 10A-10C show neutrophil uptake of albumin NPs does not alter neutrophil functions. (FIG. 10A) Number of neutrophils, concentrations of (FIG. 10B) IL-6 and (FIG. 10C) TNF-α in BALF after i.v. injection of 5% glucose (left bars), Cy5-BSA NPs (middle bars), or PEG-PS NPs (right bars) in mice 4 hours after LPS challenge (8 mg/kg). All data represent means ± SD (3-4 mice per group).

[0022] Figure 11 shows numbers of leukocytes after intravenous injection of 5% glucose (left bars), Cy5-BSA NPs (middle bars), or PEG-PS NPs (right bars) in mice 4 hours after LPS challenge (8 mg/kg). Cy5-BSA NPs and PEG-PS NPs did not change the numbers of leukocytes in BALF compared with placebo group of 5% glucose in mice with LPS challenge.

[0023] Figures 12A-12B show enhanced accumulation of albumin NPs in inflammatory lungs. (FIG. 12A) Biodistribution of albumin NPs in mice challenged with LPS. (FIG. 12B) Ratios of the amount of albumin NPs between lung and liver in mice challenged with LPS or without LPS (healthy mice). NPs were i.v. injected 4 h after LPS challenge. The samples were collected 20 h after the administration of NPs. All data represent means ± SD (n=3-4 mice per group). Statistics were performed by a two-sample student's t-test (*, $P < 0.05$). N.D., not detected.

[0024] Figures 13A-13E show neutrophil-mediated delivery of TPCA-1 mitigates acute lung inflammation/injury. (FIG. 13A) Concentrations of TPCA-1 in plasma and BALF 20 h after i.v. injection of TPCA-1 loaded BSA NPs or TPCA-1 solution. N.D., not detected. (FIG. 13B) Numbers of leukocytes and neutrophils, concentrations of (FIG. 13C) IL-6, (FIG. 13D) TNF-α and (FIG. 13E) proteins in BALF 20 h after i.v. injection of vehicle of TPCA-1 solution (far left bars), 5% glucose (second-from-left bars), TPCA-1 solution, (second-from-right bars) or TPAC-1 BSA NPs (far right bars) in mice 4 h after LPS challenge (8 mg/kg). The dose of TPCA-1 was 8 mg/kg. All data represent means ± SD (3-4 mice per group). Statistics were performed by a two-sample student's t-test (**, $P < 0.01$).

[0025] Figures 14A-14D shows HPLC chromatograph of (FIG. 14A) blank BALF, (FIG. 14B) 2 ng/mL TPCA-1 in BALF and BALF 20 h after the administration of (FIG. 14C) TPCA-1 NPs or (FIG. 14D) TPCA-1 solution. Drug was administered via tail vein in mice 4 h after LPS challenge (8 mg/kg). The internal standard (I.S.) was BMS-345541. The chromatographs indicated that TPCA-1 can be detected in BALF 20 h after the administration of TPCA-1 NPs, but not TPCA-1 solution.

[0026] Figure 15A-15D shows HPLC chromatograph of (FIG. 15A) blank plasma, (FIG. 15B) 2 ng/mL TPCA-1 in plasma and plasma 20 h after the administration of (FIG. 15C) TPCA-1 NPs or (FIG. 15D) TPCA-1 solution. Drug was administered via tail vein in mice 4 h after LPS challenge (8 mg/kg). The internal standard (I.S.) was BMS-345541. The chromatographs indicated that TPCA-1 cannot be detected in plasma 20 h after the administration of TPCA-1 NPs or TPCA-1 solution.

[0027] Figures 16A-16E show neutrophil-mediated delivery of antibiotic to infected lungs. (FIG. 16A) Number of leukocytes and neutrophils, (FIG. 16B) proteins, (FIG. 16C) IL-6, and (FIG. 16D) TNF-α in BALF after i.v. injection of 5% glucose (left bars) and Cefo-A BSA NPs (right bars) (25 mg/kg) in mice 12 h after intra-tracheal (i.t.) LPS challenge (8 mg/kg). Samples were collected 12 h later. (FIG. 16E) Total colony forming units of Pseudomonas aeruginosa in BALF after i.v. injection of 5% glucose, vehicle of Cefo-A solution, Cefo-A solution (25 mg/kg) and Cefo-A BSA NPs (25 mg/kg) in mice 12 h after Pseudomonas aeruginosa infection (1×10^6 cfu/mL, 40 μL per mouse). Samples were collected 12 h later. All data represent mean ± SD (3-4 mice per group). Statistics were performed by a two-sample student's t-test (*, $P < 0.05$).

DESCRIPTION

[0028] Various embodiments of the invention are described herein as follows. In one aspect of the present disclosure, a nanoparticle composition is provided. The nanoparticle composition comprises a drug and an albumin protein. In another aspect, a method of transporting a nanoparticle composition across a blood vessel is provided. The method comprises the step of administering the nanoparticle composition to a patient comprising the blood vessel, wherein the nanoparticle composition is transported by one or more neutrophils across a barrier of the blood vessel. In another aspect, a method of treating a disease in a patient in need thereof is provided. The method comprises the step of administering a nanoparticle composition to the patient, wherein the administration of the nanoparticle composition reduces one or more symptoms associated with the disease.

[0029] The nanoparticle composition described herein comprises a drug and an albumin protein. It is well within the ability of the skilled artisan to determine a drug that will effectively work with the described nanoparticle compositions. In some embodiments, the drug is an antibiotic. In other embodiments, the drug is an anti-inflammatory drug. In yet other embodiments, the drug is an anticancer drug.

[0030] In certain embodiments, the drug is an NF-κB inhibitor. As is known in the art, NF-κB refers to nuclear factor kappa-light-chain-enhancer of activated B cells, and is a protein complex involved in the control of DNA transcription, cytokine production, and cell survival. NF-κB can be involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens, and plays a role in regulating immune response to infection. NF-κB is involved with disease states such as cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune

development. As used herein, an "NF-κB inhibitor" refers to a drug that inhibits NF-κB. In one embodiment, the NF-κB inhibitor is TPCA-1. In various embodiments, the drug is cefoperazone acid.

[0031] As is known in the art, albumin proteins are a family of globular proteins, such as serum albumins. Albumin proteins are commonly found in blood plasma, for example plasma of humans and of bovines. In certain embodiments, the albumin protein is a bovine serum albumin (BSA) protein. In some embodiments, the BSA protein is a denatured BSA protein. In other embodiments, the albumin protein is a human serum albumin.

[0032] In various embodiments, the nanoparticle composition is conjugated to a polyethylene glycol (PEG) moiety. As used herein, the term "conjugated" refers to a complex formed between at least one nanoparticle and at least one additional agent. For example, at least one nanoparticle can be conjugated to a poly(ethylene) glycol (PEG) of varying molecular weights as known in the art. In some embodiments, the PEG may have an average molecular weight of about, e.g., 500, 1000, 2000, 3000, 3350, 3500, 4000, 4500, 5000, 6000, 8000, 10,000, or 100,000 Daltons (Da), or an average molecular weight ranging from, e.g., about 100 Da to about 100,000 Da, about 100 Da to about 6,000 Da, about 500 Da to about 5000 Da, about 1000 Da to about 4000 Da, about 2000 Da to about 4000 Da, about 2000 Da to about 6000 Da, about 1000 Da to about 10,000 Da, or about 3000 Da to about 4000 Da.

[0033] In various aspects, the nanoparticle composition can have a specified diameter. In one embodiment, the nanoparticle composition has a diameter from about 0.5 nm to about 1000 nm. In another embodiment, the nanoparticle composition has a diameter between about 10 nm to about 500 nm. In yet another embodiment, the nanoparticle composition has a diameter between about 100 nm to about 500 nm. In another embodiment, the nanoparticle composition has a diameter between about 200 nm to about 400 nm. In certain embodiments, the nanoparticle composition has a diameter between about 100 nm to about 150 nm.

[0034] In one embodiment, the nanoparticle composition has a diameter of about 100 nm. In another embodiment, the nanoparticle composition has a diameter of about 200 nm. In yet another embodiment, the nanoparticle composition has a diameter of about 300 nm. In another embodiment, the nanoparticle composition has a diameter of about 400 nm. In yet another embodiment, the nanoparticle composition has a diameter of about 500 nm.

[0035] In another aspect, a method of transporting a nanoparticle composition across a blood vessel is provided. The method comprises the step of administering the nanoparticle

composition to a patient comprising the blood vessel, wherein the nanoparticle composition is transported by one or more neutrophils across a barrier of the blood vessel. The previously described embodiments of the nanoparticle composition are applicable to the method of transporting a nanoparticle composition across a blood vessel described herein.

[0036] In certain embodiments, the transporting across the blood vessel is via a transcellular pathway. In some embodiments, the transporting across the blood vessel is via a neutrophil transmigration pathway. In certain aspects, the neutrophil is a polymorphonuclear neutrophil. As is known in the art, polymorphonuclear neutrophils (PMNs) are circulating blood leukocytes that provide the first-line defense against infection and are potent effectors of inflammation. Furthermore, PMNs respond to and produce cytokines and thus can modulate the balance between humoral and cell-mediated immunity by contributing to the promotion of a T_H1 or T_H2 response.

[0037] In some embodiments, the neutrophil is an activated neutrophil. Activated neutrophils produce reactive oxygen species (ROS), release proteolytic enzymes, and express higher and functional levels of the β2 integrin Mac-1 (or CD11b) on their cell surface, all of which can affect the hemostatic system and induce a prothrombotic condition. In some aspects, neutrophil comprises a Fcγ receptor. In some aspects, the nanoparticle composition is internalized into the neutrophil.

[0038] In various embodiments, the barrier of the blood vessel is an endothelial vessel barrier. In some embodiments, the blood vessel is a lung blood vessel.

[0039] In some embodiments, the transporting across the blood vessel enhances the delivery of the drug to a desired site. For example, a desired site for which drug delivery is advantageously enhanced may be a site of inflammation, a site of infection, a tumor site, or a cancerous cell site.

[0040] In some aspects, the method further comprises administering a neutrophil migration drug to the patient. A neutrophil migration drug as used herein enhances transportation of neutrophils across the barrier of the blood vessel. In some embodiments, the neutrophil migration drug is PD98059.

[0041] In another aspect, a method of treating a disease in a patient in need thereof is provided. The method comprises the step of administering a nanoparticle composition to the patient, wherein the administration of the nanoparticle composition reduces one or more

symptoms associated with the disease. The previously described embodiments of the nanoparticle composition are applicable to the method of treating a disease in a patient in need thereof described herein. The previously described embodiments of the method of transporting a nanoparticle composition across a blood vessel are applicable to the method of treating a disease in a patient in need thereof described herein.

[0042] In certain aspects, the disease is an inflammatory disease. In some embodiments, the inflammatory disease is an acute inflammatory disease. In other embodiments, the inflammatory disease is a chronic inflammatory disease. In yet other embodiments, the inflammatory disease is cancer. In some embodiments, the inflammatory disease is sepsis. In other embodiments, the inflammatory disease is a lung injury. In one embodiment, the lung injury is an acute lung injury. In another embodiment, the lung injury is a chronic lung injury.

[0043] In some aspects, the disease is an infection. In some embodiments, the infection is a bacterial infection. In other embodiments, the infection is a viral infection. In yet other embodiments, the infection is a fungal infection.

[0044] In certain embodiments, the administration is a parenteral administration. Suitable routes for such parenteral administration include intravenous, intraarterial, intraperitoneal, intrathecal, intradermal, epidural, intracerebroventricular, intraurethral, intrasternal, intracranial, intratumoral, intramuscular and subcutaneous delivery. In some embodiments, the parenteral administration is an intravenous administration. Suitable means for parenteral administration include needle (including microneedle) injectors, needle-free injectors and infusion techniques.

[0045] In other embodiments, the administration is an oral administration. The term "oral administration" refers to the provision of a composition via the mouth through ingestion, or via some other part of the gastrointestinal system including the esophagus. Examples of oral dosage forms include tablets (including compressed, coated or uncoated), capsules, hard or soft gelatin capsules, pellets, pills, powders, granules, elixirs, tinctures, colloidal dispersions, dispersions, effervescent compositions, films, sterile solutions or suspensions, syrups and emulsions and the like.

[0046] In certain aspects, a therapeutically effective amount of the nanoparticle composition is administered to the patient. As used herein, the term “therapeutically effective amount” refers to an amount which gives the desired benefit to a patient and includes both treatment and prophylactic administration. The amount will vary from one patient to another and will depend upon a number of factors, including the overall physical condition of the patient and the

underlying cause of the condition to be treated. As used herein, the term "patient" refers to an animal, for example a human.

[0047] A "therapeutically effective amount" can be determined by a skilled artisan, and can be calculated based on the amount of drug in the nanoparticle composition, on the amount of albumin in the nanoparticle composition, or both. In some embodiments, the therapeutically effective amount of the nanoparticle composition is administered to the patient at a dose of about 0.001 to about 1000 mg. In one embodiment, the therapeutically effective amount of the nanoparticle composition is administered to the patient at a dose of about 0.001 to about 100 mg. In another embodiment, the therapeutically effective amount of the nanoparticle composition is administered to the patient at a dose of about 0.01 to about 100 mg. In yet another embodiment, the therapeutically effective amount of the nanoparticle composition is administered to the patient at a dose of about 0.1 to about 100 mg. In one embodiment, the therapeutically effective amount of the nanoparticle composition is administered to the patient at a dose of about 0.1 to about 10 mg. In one aspect of the described method, the disease is cancer and wherein the method is cancer immunotherapy.

[0048] The following examples as well as the figures are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples or figures represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

[0049] Examples 2-9 utilize the following exemplary materials and methods.

[0050] Lipopolysaccharide (LPS), bovine serum albumin (BSA) and BMS-345541 were obtained from Sigma (St. Louis, MO). Cefoperazone acid (Cefo-A) was obtained from Santa Cruz Biotechnology (Dallas, TX). Glutaraldehyde was obtained from Electron Microscopy Sciences (Hatfield, PA). Alexa Fluor-488 and Alexa Fluor-647-labeled anti-mouse Gr-1(Ly-6G/Ly-C6) and Ultra-LEAF™ Purified anti-mouse Ly-6G/Ly-6C (Gr-1) antibody (Clone: RB6-8C5) were purchased from Biolegend (San Diego, CA). Carboxylated polystyrene fluorescent yellow-green NPs (100 nm in a diameter; 2%, w/v; excitation/emission: 505 nm/515 nm) were

obtained from Invitrogen (Grand Island, NY). EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) and Sulfo-NHS (N-hydroxysulfo succinimide) were obtained from Pierce (Rockford, IL). mPEG-NH_2 (5,000 dalton) was obtained from Laysan Bio (Arab, AL). MES ((2-N-morpholino) ethanesulfonic acid) was from Fisher Scientific (Pittsburg, PA). TPCA-1 was obtained from Medchemexpress (Monmouth Junction, NJ). Freeze-dried *pseudomonas aeruginosa* (*P. aeruginosa*) (ATCC 29260) was obtained from ATCC (Manassas, VA).

[0051] **Preparation of Cy5-BSA NPs and TPCA-1 or cefoperazone acid loaded BSA NPs (TPCA-1 or Cefo-A BSA NPs).** BSA was labeled with Cy5 NHS ester (Lumiprobe, Hallandale Beach, FL) according to manufacturer's protocol. Cy5-BSA NPs (Cy5-BSA/BSA, 1:1, w/w) were prepared by the desolvation technique. BSA was first dissolved at the concentration of 20 mg/mL in deionized water. Afterwards, 0.2 mL DMSO was added to 1 mL of the BSA solution, which was stirred at 1600 rpm. Fifteen minutes later, the solution was quickly mixed with 3.5 mL of ethanol under stirring (600 rpm) at room temperature. To make TPCA-1 or Cefo-A BSA NPs, 1 mL of 20 mg/mL BSA solution was incubated with 4 mg of TPCA-1 or 0.5mg Cefo-A dissolved in 0.2 mL DMSO for 15 minutes, and then mixed with 3.5 mL of ethanol. To form stable albumin NPs with or without drug, 1 hour later BSA molecules were crosslinked by adding 80 μL of 2% glutaraldehyde into the suspension. The suspension was stirred overnight at room temperature and centrifuged at 20,000 g for 30 minutes at 4 °C. The nanoparticle pellet was centrifuged for three times to remove organic solvents and the un-encapsulated drug, and then was re-suspended in water or 5% glucose for experiments.

[0052] To measure the drug content of BSA NPs, the concentrations of TPCA-1 or Cefo-A were determined by a Waters HPLC with 2690 Separations Module, 486 UV detector and Ultra C18 column, 4.6 × 250 mm, 5 μm (Restek, Bellefonte, PA). The flow rate was 1 mL and the injection volume was 5 μL. For TPCA-1, the mobile phase was methanol/water, 65: 35, v/v and the detection wavelength was 310 nm. The standards were prepared in methanol and the linear range was between 1 and 25 μg/mL ($R = 0.999$). For Cefo-A, the initial mobile phase was methanol/0.005% acetic acid aqueous solution, 10: 90, v/v, which gradually changed to 90: 10, v/v at 10 minutes. At 10.01 minutes the ratio of methanol/aqueous solution was decreased to 10% for 5 minutes. The standards were prepared in deionized water and the linear range was between 1 and 25 μg/mL ($R = 0.999$). Furthermore, NPs suspension was dried by lyophilization and weighed. The drug content of TPCA-1 in NPs was calculated with the following equation: drug content = (drug used–unloaded drug)/dried BSA NPs. Finally, BSA NPs containing 25 ± 1.3 wt% of TPCA-1 and 3.2 ± 0.3 wt% of Cefo-A, respectively, were obtained. The particle sizes were measured by Malvern Zetasizer Nano ZS90 (Westborough, MA).

[0053] **Preparation of PEG-Polystyrene NPs (PEG-PS NPs).** To covalently conjugate mPEG-NH_2 5k to polystryrene COOH-NPs (PS COOH-NPs), the carboxyl-amine reaction was utilized. The COOH groups on PS NPs were first activated. In detail, 0.3 mL of original particle solution (20 mg/mL) in 2.7 mL of MES buffer (50 mM, pH = 6) was mixed with 75 μL of EDC (80 mg/mL) and 75 μL of Sulfo-NHS (160 mg/mL) for reaction of 15 minutes. The mixture was washed with PBS buffer (pH = 7.4) and centrifuged using Amicon Ultra-4 100k Centrifugal Filter Units (EMD Millipore, Billerica, MA) three times. After activation of NPs, 3 mL of 20 mg/mL mPEG-NH_2 solution was added to the activated particle solution and allowed to react for 2 hours at room temperature. The mixture was washed with 5% glucose and centrifuged three times using the same Filter Units as described above. After reaction, the particle sizes increased from 109 nm to 116.7 nm and the zeta potentials decreased from -40.7 mV to -27.7 mV in terms of PS COOH-NPs and PS PEG-NPs, indicating that we successfully conjugated PEG molecules to NPs.

[0054] **Mice.** Adult CD1 mice (22-26 g, 4-5 weeks) were obtained from Harlan Labs (Madison, WI). The mice were maintained in polyethylene cages with stainless steel lids at 20 °C with a 12 hour light/dark cycle and covered with a filter cap. Animals were fed with food and water *ad lib*. The Washington State University Institutional Animal Care and Use Committee approved all animal care and experimental protocols used in these studies. All experiments were made under anesthesia using intraperitoneal (i.p.) injection of the mixture of ketamine (120 mg/kg) and xylazine (6 mg/kg) in saline.

[0055] **Intravital Microscopy of Live Mouse Cremaster Venules.** Using intravital microscopy, it can be visualized in real-time that neutrophils take up albumin NPs and transport them across endothelial vessel walls moving to inflammation sites in live mouse cremaster venules. TNF-α (500 ng in 250 μL saline) was intrascrotally injected into the mice. At 2 hours after TNF-α injection, the mice were anesthetized with i.p. injection of a mixture of ketamine (120 mg/kg) and xylazine (6 mg/kg), and maintained at 37 °C on a thermo-controlled rodent blanket. A tracheal tube was inserted and a right jugular vein was cannulated for injection of NPs, antibodies or drugs. After the scrotum was incised, the testicle and surrounding cremaster muscles were exteriorized onto an intravital microscopy tray. The cremaster preparation was perfused with thermo-controlled (37 °C) and aerated (95% N_2, 5% CO_2) bicarbonate-buffered saline throughout the experiment. Images were recorded using a Nikon A1R$^+$ laser scanning confocal microscope with a resonant scanner. To study neutrophil uptake of albumin NPs, 30 minutes after i.v. injection of Alexa Fluor 488-labled Gr-1 antibody (1.5 μg/mouse) and Cy5-BSA NPs (80 μg/mouse) into the TNF-α-treated mouse, water immersion objective with

NA=1.1 was used to image cremaster venules. Two lasers at 488 nm and 640 nm simultaneously excited cremaster tissues to image neutrophils and albumin NPs at 10 frames/s for 512x512 pixels. Images were analyzed using Nikon software.

[0056] **LPS challenge and Neutrophils depletion.** After the mice were anesthetized, they were placed in a supine position head up on a board tilted at 15°. Afterwards, 8 mg/kg of LPS from Escherichia coli (serotype 0111.B4, Sigma Aldrich, St. Louis, MO) in 50 μL of Hank's Balanced Salt Solution (HBSS) were nebulized into the pulmonary alveoli with a FMJ-250 High Pressure Syringe (Penn-Century, Wyndmoor, PA). Mice were held upright for 2 minutes after the administration. For neutrophil depletion, after LPS challenge, the mice were i.p. injected with 0.5 mg of Ultra-LEAF™ Purified anti-mouse Ly-6G/Ly-6C (Gr-1) antibody.

[0057] **Bronchoalveolar Lavage fluid (BALF) , blood, plasma and organs collection.** Approximately 4 hours after LPS challenge, 200 μL of 16 mg/kg of PEG-PS NPs in 5% glucose, 16 mg/kg of Cy5-BSA NPs in 5% glucose, 16 mg/kg of Cy5-BSA NPs in 5% glucose + neutrophils depletion, 8 mg/kg of TPCA-1 in 5% DMSO (v/v), 10% Tween 20 (v/v), 5% glucose solution or TPCA-1 BSA NPs with TPCA-1 at 8 mg/kg in 5% glucose were i.v. injected into the mice *via* tail vein, respectively. At predetermined time points, mouse BAL fluid (BALF) was collected by inserting a needle into the upper trachea. Cefo-A BSA NPs with Cefo-A at 25 mg/kg in 200 μL 5% glucose was i.v. injected to the mice at 12 hours after LPS challenge. BALF were then harvested at 12 hours after NPs were administered. Lavage was performed by introducing three sequential 0.9 mL of HBSS into the lungs and BALF was carefully withdrew. The BALF was centrifuged at 350 g for 10 minutes. The supernatant of BALF was collected and stored at -20 °C. Cells were resuspended in 1 mL red blood cell (RBC) lysis buffer (Qiagen Sciences, Germantown, MD) for 30 minutes at room temperature. The cell suspensions were then washed with 1 mL HBSS and centrifuged for three times. Afterwards, the cell suspensions were resuspended in 200 μL HBSS for the quantitation of PEG-PS NPs and Cy5-BSA NPs in the cells. In different groups, the cell pellet was resuspended in 1 mL HBSS, 10 μL of which was employed for counting the number of cells in a hemocytometer and 200 μL of which was used to prepare the slide smears by 7620 Cytopro Cytocentrifuge (ELITech, Princeton, NJ). The smears were stained with Differential Quick Stain Kit (Polysciences, Warrington, PA) and then leukocytes and neutrophils were quantified under a microscope. Afterwards, the cell suspension was concentrated to 400 μL for flow cytometry and confocal microscopy. Blood was collected into the heparin tubes by cardiac puncture. The neutrophils in blood were isolated by Pluriselect anti-mouse-Ly6G S-pluribeads according to the

manufacturer's protocol (Pluriselect, Spring Valley, CA). The plasma was harvested and stored at -20 °C after the blood was centrifuged at 1,500 g for 20 min. After the depletion of neutrophils, blood was also treated with RBC lysis buffer and subject to hemocytometer and Differential Quick Stain Kit. After the administration of the Cy5-BSA NPs, various organs were collected from the mice with and without LPS challenge and stored at -80 °C.

[0058] **Determination of NPs in BALF, plasma, blood and organs.** The concentrations of PEG-PS NPs (Excitation 485 nm and emission 515 nm) in BALF supernatant, BALF cells and plasma, and the concentrations of Cy5-BSA NPs (Excitation 645 nm and emission 675 nm) in BALF supernatant, BALF cells, plasma, blood and various organs were measured by Synergy Neo fluorescence plate reader (BioTek, Winooski, VT). For PEG-PS NPs and Cy5-BSA NPs in BALF and plasma, standards were prepared by adding the NPs to BALF supernatant, BALF cells in 200 μL HBSS after the treatment of RBC lysis buffer and plasma collected from the mice 2, 10 and 20 hours after LPS challenge without the administration of NPs. The linear range was from 0.02 to1 μg/mL ($R = 0.989$) for PEG-PS NPs and 0.04 to 6 μg/mL ($R = 0.992$) for Cy5-BSA NPs in BALF supernatant and cells. In plasma, the linear range was between 0.2 and 1 μg/mL ($R = 0.991$) for PEG-PS NPs and 0.8 to 6 μg/mL ($R = 0.993$) for Cy5-BSA NPs. In blood, standards were prepared by adding the Cy5-BSA NPs to blood collected from the mice 20 hours after LPS challenge without the administration of NPs. The linear range was between 1 to 10 μg/mL ($R = 0.985$) for Cy5-BSA NPs. In terms of Cy5-BSA NPs in various organs, standards were prepared by adding the Cy5-BSA NPs to the blank organ collected from the mice at 20 hours with or without LPS challenge. Afterwards, the organs were homogenized with 4 parts (w/w) of PBS buffer to obtain the pipettable homogenate. Finally, 100 mg of the homogenate was subject to the fluorescence measurement mentioned above with the linear range of 4 to 100 μg/mL ($R = 0.988$). Samples were diluted when they were beyond the calibration range.

[0059] **Determination of TPCA-1 in BALF and plasma.** Both 25 μL of various TPCA-1 solutions and 25 μL of 4 μg/mL BMS 345541 internal standard solution in methanol were added to 300 μL of plasma or 500 μL of BALF from the mice 20 hours after LPS challenge without the administration of NPs. In terms of the plasma and BALF samples from the mice administered with TPCA-1 NPs, only 50 μL methanol was added. The samples were then vortexed for 0.5 minutes before 2 mL of chloroform was added. After the tubes were vortexed for 5 minutes, they were centrifuged at 3,000 g for 20 min. Afterwards, organic layers were transferred to clean tubes and evaporated to dryness at room temperature under a stream of air.

Residues were reconstituted in 50 μL of methanol/0.0025% (v/v) acetic acid aqueous solution, 30: 70, v/v, which were briefly vortex-mixed and then 20 μL was injected into the HPLC system with the same flow rate and detection wavelength as described previously. The initial mobile phase was methanol/0.0025% acetic acid aqueous solution, 30: 70, v/v, which gradually changed to 90: 10, v/v at 8 min. At 10.01 minutes the ratio of methanol/aqueous solution was decreased to 30% for 5 min. Quantitation of TPCA-1 was based on calibration curves of peak area ratio (drug/internal standard) *versus* concentration.

[0060] **Determination of IL-6, TNF-α and total protein in BALF.** The concentrations of IL-6 and TNF-α in BALF were determined with ELISA MAX Deluxe Sets (Biolegend, San Diego, CA) and the protein in BALF was measured by Pierce BCA protein Assay Kit (Thermo Scientific, Rockford, IL) according to manufacturer's protocol.

[0061] **Flow cytometry.** The neutrophils from blood and the BALF cells were washed with 3 mL of 5% BSA HBSS and centrifuged at 350 g for 5 minutes three times, which was finally resuspended in 400 μL of 5% BSA HBSS. The cell suspension was incubated with 1 μg of Alexa Fluor 488-conjugated anti-mouse Gr-1 antibody for Cy5-BSA NPs groups and Alexa Fluor-647-labeled anti-mouse Gr-1 antibody for PEG-PS NPs groups in the dark for 20 min, followed by washing with 3 mL of 0.1% BSA HBSS under centrifugation for three times. Cells were then resuspended in 400 μL 0.1% BSA HBSS and analyzed by Accuri C6 flow cytometer (BD Biosciences, San Jose, CA).

[0062] **Confocal microscope.** The cell suspensions were fixed with 2 mL of 4% paraformaldehyde overnight, which was then resuspended in 200 μL of HBSS. Smears on slide were prepared by the same methods as described above. After one drop of Prolong Gold antifade reagent with DAPI (Invitrogen, Eugene, OR) was added on the cells, a cover slide was applied. Approximately 4 hours later, the cells were observed by Olympus Fluoview FV1000 confocal microscope (Center Valley, PA).

[0063] ***P. aeruginosa* culture.** The freeze-dried bacteria was streaked on the sheep blood agar plates (Hardy diagnostics, Santa Maria, CA) and grew at 37 °C for 15 hours in an incubator. The bacteria were then scratched from the plate and re-suspended in PBS, pH 7.4. The concentrations of bacteria were determined by counting colony forming units (CFU) of diluted bacteria suspension on the sheep blood agar plates after incubation 37 °C for 15 hours.

[0064] **Lung infection of *P. aeruginosa*.** Anesthetized mice were first placed in a supine position head up on a board tilted at 15°, and then 1×10^6 CFU of *P. aeruginosa* in 40 μL of

PBS, pH 7.4 were instilled to the trachea. Mice were held upright for 2 minutes after the administration.

[0065] **Bacteriological assessment of BALF of infected lungs.** Approximately 12 hours after *P. aeruginosa* infection, 200 μL of Cefo-A solution of 5% DMSO (v/v), 10% Tween (v/v) 20 and 5% glucose (w/v) (25 mg/kg) or Cefo-A BSA NPs in 5% glucose with Cefo-A at 25mg/kg was injected *via* tail vein. The BALF were collected 12 hours after the drug administration as mentioned above. The CFU of *P. aeruginosa* in BALF was counted on the sheep blood agar plates after incubation 37 °C for 15 hours.

Example 2

Activated neutrophils transport albumin NPs across blood vessel barrier

[0066] Intravital microscopy of mouse cremaster venules was performed to real-time visualize whether neutrophils can transport albumin NPs across endothelial vessels into inflamed tissues. Approximately 2 hours after intra-scrotal injection of 0.5 μg TNF-α (tumor necrosis factor), neutrophils were activated and adherent to the endothelium of cremaster venules, and were ready to migrate from bloodstream to inflamed tissues. Approximately 30 minutes after i.v. injection of both Alexa-Fluor-488-labeled mouse anti-Gr-1 to mark neutrophils and bovine serum albumin (BSA) NPs conjugated with Cy5 (Cy5-BSA NPs) (the size is 130 nm, Figures 1A and 1B), the neutrophils were imaged in live mouse cremaster venules using intravital microscopy. The albumin NPs were internalized by adherent neutrophils and the neutrophils were moving into the muscle (Figure 2B). Approximately 30 minutes later, imaging was performed again and it was determined that some neutrophils containing NPs migrated across the vessels. The intravital images clearly demonstrated that activated neutrophils can transport albumin NPs across the blood vessel barrier.

[0067] Fluorescent dyes were physically incorporated in albumin NPs and found that the albumin NPs can be specifically internalized by activated neutrophils using intravital microscopy. The nanoparticle uptake is mediated *via* neutrophil Fcγ receptors interacting with denatured albumin after albumin nanoparticle formation. When NPs were coated with natural albumin protein, neutrophils did not internalize the NPs. It was determined that albumin nanoparticle uptake is independent of fluorescent labeling on their surface. In the instant example, fluorescent dyes were conjugated to BSA first and then mixed with non-fluorescent BSA to fabricate albumin NPs showing the same neutrophil uptake as those of Cy5-loaded and Cy5 coated albumin NPs. Therefore, the nanoparticle uptake by neutrophils is mainly

determined by denaturation of albumin rather than by fluorescent dyes. The size and zeta potential of albumin NPs made of fluorescently-labeled albumin or loaded with drugs using dynamic light scattering were characterized (Figure 1A-1C). The labeling and drug loading did not affect the properties of albumin NPs significantly. Moreover, the particle sizes did not change upon a series of dilution of NPs in 5% glucose and PBS, pH 7.4 (Figure 1D) and no free BSA was detected when the NPs were diluted in serum (supporting information), suggesting that our NPs are stable, which would be attributed to the fact that albumin protein was crosslinked after it formed a particle.

Example 3

Neutrophils mediate delivery of albumin nanoparticles across lung blood-vessel barrier

[0068] To further confirm the finding that neutrophils can carry albumin NPs across the vessel barrier in a large tissue *in vivo*, an acute lung inflammation model in mice was evaluated. The lung has unique features with many tiny air sacs (called alveoli) surrounded by blood capillaries to form an interface between circulation and airspace. When there is bacterial or viral infection in the airspace, neutrophils are capable of migrating from bloodstream to alveoli passing through the endothelial and epithelial barriers and accumulating in a distal lung airspace. Therefore, the mouse model of acute lung inflammation can be utilized for the instant example.

[0069] Approximately 4 hours after intra-tracheal (i.t.) administration of lipopolysaccharide (LPS) (8mg/kg) to the lung, neutrophils were activated and adherent to vessel walls, preparing to transmigrate into the lung airspace. Afterwards, albumin NPs (the size is 130 nm, Figures 1A-1D) were injected i.v. to the mouse. At 2, 10 and 20 hours after nanoparticle injection, lung bronchoalveolar lavage fluid (BALF) and blood were collected. The cells were isolated and stained with mouse anti-Gr-1 antibody to mark neutrophils. The confocal imaging (Figures 2C and 2D) showed that transmigrated neutrophils internalized albumin NPs and the number of neutrophils containing NPs temporally increased.

[0070] To determine the percentage of lung infiltrated neutrophils which have internalized albumin NPs, flow cytometry was performed to analyze the BALF collected at 2, 10 and 20 hours after i.v. injection of NPs. Control, healthy mice (without LPS treatment) did not show the infiltration of neutrophils (Figure 3A). After LPS challenge, the cell population became primarily neutrophils, and the percentage of neutrophils increased with time (Figure 4). After i.v. injection of albumin NPs, the infiltrated neutrophils were divided to two populations: with

and without the NPs (Figure 2E), but the percentage of totally infiltrated neutrophils was similar to the control (Figure 4) at each time point. Approximately 2 hours after albumin nanoparticle injection, 6% of infiltrated neutrophils internalized albumin NPs and the number increased to 30% after 20 hours (Figure 2F), which is consistent with the neutrophil transmigration process.

[0071] At different time points, neutrophils were isolated in peripheral blood in LPS-challenged mice, finding that the percentage of neutrophils containing the NPs decreased with time (Figure 2G and Figure 5). The finding that the percentage of NPs contained neutrophils decreased with time in blood but accordingly it increased in BALF (Figures 2F and 2G), is correlated with the neutrophil transmigration which mediates the transport of albumin NPs. To address whether activation of neutrophils is required for nanoparticle uptake, albumin NPs were administrated in healthy mice (without LPS challenge) *via* a tail vein. It was determined that resting neutrophils did not internalize albumin NPs (Figure 2G). The observation showed that only activated neutrophils (under inflammatory conditions) internalized the albumin NPs. This result was also demonstrated when we studied the uptake of albumin NPs by neutrophils in mouse cremaster venules using intravital microscopy.

Example 4

LPS-induced lung permeability does not contribute to the transport of albumin NPs into lungs

[0072] To rule out the possibility that NPs would first pass through leaky vessels induced by LPS and then the infiltrated neutrophils internalize them in the lung airspace, the concentrations of NPs in the plasma and BALF in a LPS-induced acute lung inflammation model were measured. PEG-coated-yellow-green polystyrene NPs (PEG-coated NPs) were used as control and were produced with a similar size as albumin NPs (Figures 1A-1D). The protein influx into mouse lungs represents an increase of lung vessel permeability. Therefore, a time course of lung permeability after LPS challenge and i.v. injection of albumin NPs or PEG-coated NPs was evaluated by measuring the protein concentrations in BALF. The results indicated that the LPS challenge increased the lung permeability with time and the injection of NPs did not affect the lung permeability (Figure 6A). Approximately 2, 10, and 20 hours after injection of the PEG-coated NPs (Figure 6B) (Figure 3B for healthy mice and Figure 7 for the control without NPs), it was determined that less than 1% of neutrophils containing PEG-coated NPs in BALF (Figure 6C), which is unlikely that PEG-coated NPs were internalized by neutrophils, as evident in the confocal imaging (Figure 8).

[0073] The dynamics of PEG-coated NPs and albumin NPs in the plasma and BALF were also evaluated. The PEG-coated NPs had much longer circulation than albumin NPs, which were even observed at 20 hours after i.v. injection; however, the albumin NPs totally disappeared after 10 hours (Figure 6D). In BALF, the PEG-coated NPs were not detected at 2, 10 and 20 hours after their injection, but the albumin NPs were observed at 10 and 20 hours (Figure 6E). Consistent with the flow cytometry and confocal imaging (Figures 2A-2G), the results indicated that the transport of albumin NPs across lung blood vessel walls is an active process mediated by neutrophils rather than the nanoparticle diffusion through the leaky vasculature.

Example 5

Neutrophil depletion abolishes the delivery of albumin NPs across blood vessel barrier

[0074] To support the conclusion that neutrophils are a carrier to deliver albumin NPs across the blood vessel barrier, the depletion of neutrophils using mouse anti-Gr-1 antibody was performed. To assure the similar lung permeability between before and after the neutrophil depletion, experiments were optimized.

[0075] Approximately 4 hours after LPS challenge, anti-Gr-1 antibody and i.v. injected albumin NPs were intraperitoneally (i.p.) injected into mice. Approximately 20 hours later, the lung permeability was measured. It was observed that the administration of anti-Gr-1 antibody did not significantly change the lung permeability induced by LPS (Figure 9A). When the number of neutrophils were counted, it was determined that the neutrophils in peripheral blood and BALF were depleted (Figure 9B). When albumin NPs in BALF were analyzed, they were not detected either. However, without injection of anti-Gr-1, the albumin NPs were observed in the BALF (Figure 9C). The result clearly demonstrated that albumin NPs accumulated in the lungs is mediated by the neutrophil transmigration pathway.

Example 6

Uptake of albumin NPs does not change the functions of neutrophils

[0076] Next, whether the internalization of albumin NPs will affect the neutrophil mobility and activation was evaluated. After injection of NPs (PEG-coated NPs or Cy5-BSA NPs), the number of infiltrated neutrophils (Figure 10A) and total leukocytes (Figure 11) did not significantly change compared with the control. Nanoparticle uptake also did not affect the cytokine release, such as IL-6 and TNF-α (Figures 10B and 10C), indicating that the nanoparticle internalization did not further promote the activation of neutrophils. Moreover,

neutrophil uptake of albumin NPs did not alter the lung integrity because the protein concentration in lungs was similar to the PEG-coated NPs and the control (Figure 6A). Together, the results showed that neutrophils are a vehicle that chaperones albumin NPs across blood vessels and deposit them in targeted locations.

Example 7

Increased accumulation of albumin NPs in inflammatory lungs

[0077] Although neutrophils are capable of mediating the delivery of albumin NPs into inflammatory lungs, it is not clear whether the accumulation of albumin NPs can be enhanced compared with that in healthy mice. After i.v. injection of albumin NPs in LPS-challenged mice, the albumin NPs mainly distributed in the liver, but they were also observed in the lung (Figure 12A). Quantification of the nanoparticle concentration ratio between lung and liver determined that the ratio in LPS-challenged mice was significantly higher than that in healthy ones (Figure 12B). The result is consistent with the active delivery of albumin NPs mediated by the neutrophil transmigration.

Example 8

TPCA-1-loaded albumin NPs alleviate acute lung inflammation/injury

[0078] Acute lung injury (ALI), and its most severe form, acute respiratory distress syndrome (ARDS), cause 40% mortality in approximately 200,000 patients annually in the United States. The pathological underlying of ALI is primarily linked to cytokine storms produced by resident lung macrophages. The NF-κB pathway plays a central role in the production of cytokine storms. Thus, it was investigated whether neutrophils could mediate the delivery of albumin NPs loaded with a NF-κB inhibitor to improve the treatment of acute lung inflammation and injury.

[0079] TPCA-1 (2-[(Aminocarbonyl)amino]-5-(4-fluorophenyl)-3-thiophenecarboxamide), a NF-κB inhibitor, was loaded into albumin NPs and quantified the TPCA-1 loading efficiency using high performance liquid chromatography (HPLC). The TPCA-1 concentrations in BALF and plasma were measured 20 hours after injection of TPCA-1-loaded albumin NPs and free TPCA-1, respectively. It was found that TPCA-1 was detected in the BALF, but not in the plasma when mice were treated with TPCA-1-loaded albumin NPs (Figure 13A and Figures 14A-14D and Figures 15A-15D). In contrast, TPCA-1 was detected neither in the BALF nor in the plasma for TPCA-1 solution (Figure 13A and Figures 14A-14D and Figures 15A-15D).

These results demonstrated that drug-loaded albumin NPs can be delivered across the blood vessel barrier and accumulate in the lung tissues.

[0080] To evaluate the therapeutic effect, inflammatory parameters (neutrophil infiltration and cytokine release (IL-6 and TNF-α)) after the treatment with TPCA-1-loaded albumin NPs were compared to those after the administration of free drug solution (Figures 13B-13D). The results indicated that the TPCA-1-loaded albumin NPs dramatically attenuated the lung inflammation (Figures 13B-13D) and lowered the lung permeability compared with the free drug (Figure 13E), representing that the albumin NPs can improve the lung integrity to prevent lung edema.

Example 9

Antibiotic-loaded albumin NPs impair bacterial infection

[0081] *P. aeruginosa* is one of the most recalcitrant pathogens and a leading cause of acute pneumonia. Thus, there is a need to specifically target the pathogenic locations, such as in the lung. Using the albumin NPs loaded with cefoperazone acid (Cefo-A), a broad-spectrum antibiotic, improvement of the treatment of lung infection was evaluated. In control experiments, the leukocyte infiltration, inflammation factors (IL-6 and TNF-α), and lung permeability were analyzed after i.v. injection of the Cefo-A loaded albumin NPs in the mice with LPS-induced lung inflammation. The comparison with vehicle group of 5% glucose showed that the Cefo-A-loaded albumin NPs did not affect the neutrophil mobility and activation and the lung integrity (Figures 16A-16B). Furthermore, Cefo-A-loaded albumin NPs did not attenuate lung inflammation (Figures 16C-16D). The results suggest that Cefo-A-loaded albumin NPs did not affect the functions of neutrophils and inflammatory response of the mice to LPS challenge. Cefo-A does not have the anti-inflammatory effect, so it is not surprising that the impairment of inflammation induced by LPS after the administration of Cefo-A-loaded albumin NPs was not observed.

[0082] When delivering the Cefo-A-loaded albumin NPs in *P. aeruginosa*-infected lungs, NPs dramatically declined bacterial proliferation by three folds compared with free Cefo-A in solution (Figure 16E). These results provide further evidence of neutrophils as a carrier to mediate the delivery of therapeutic agents into targeted tissues.

WHAT IS CLAIMED IS:

1. A nanoparticle composition comprising a drug and an albumin protein.

2. The nanoparticle composition of claim 1 wherein the drug is an antibiotic.

3. The nanoparticle composition of claim 1 wherein the drug is an anti-inflammatory drug.

4. The nanoparticle composition of claim 1 wherein the drug is an anticancer drug.

5. The nanoparticle composition of claim 1 wherein the drug is an NF-κB inhibitor.

6. The nanoparticle composition of claim 5 wherein the NF-κB inhibitor is TPCA-1.

7. The nanoparticle composition of claim 1 wherein the drug is cefoperazone acid.

8. The nanoparticle composition of claim 1 wherein the albumin protein is a bovine serum albumin (BSA) protein.

9. The nanoparticle composition of claim 8 wherein the BSA protein is a denatured BSA protein.

10. The nanoparticle composition of claim 1 wherein the nanoparticle composition is conjugated to a polyethylene glycol moiety.

11. The nanoparticle composition of claim 1 wherein the nanoparticle composition has a diameter between about 100 nm to about 150 nm.

12. A method of transporting a nanoparticle composition across a blood vessel, said method comprising the step of administering the nanoparticle composition to a patient comprising the blood vessel, wherein the nanoparticle composition is transported by one or more neutrophils across a barrier of the blood vessel.

13. The method of claim 12 wherein the transporting across the blood vessel is via a transcellular pathway.

14. The method of claim 12 wherein the transporting across the blood vessel is via a neutrophil transmigration pathway.

15. The method of claim 12 wherein the neutrophil is a polymorphonuclear neutrophil.

16. The method of claim 12 wherein the neutrophil is an activated neutrophil.

17. The method of claim 12 wherein the neutrophil comprises a Fcγ receptor.

18. The method of claim 12 wherein the nanoparticle composition is internalized into the neutrophil.

19. The method of claim 12 wherein the barrier of the blood vessel is an endothelial vessel barrier.

20. The method of claim 12 wherein the blood vessel is a lung blood vessel.

21. The method of claim 12 wherein the nanoparticle composition comprises a drug and an albumin protein.

22. The method of claim 21 wherein the drug is an antibiotic.

23. The method of claim 21 wherein the drug is an anti-inflammatory drug.

24. The method of claim 21 wherein the drug is an anticancer drug.

25. The method of claim 21 wherein the drug is an NF-κB inhibitor.

26. The method of claim 25 wherein the NF-κB inhibitor is TPCA-1.

27. The method of claim 21 wherein the drug is cefoperazone acid.

28. The method of claim 21 wherein the albumin protein is a bovine serum albumin (BSA) protein.

29. The method of claim 28 wherein the BSA protein is a denatured BSA protein.

30. The method of claim 21 wherein the nanoparticle composition is conjugated to a polyethylene glycol moiety.

31. The method of claim 21 wherein the nanoparticle composition has a diameter between about 100 nm to about 150 nm.

32. The method of claim 21 wherein the transporting across the blood vessel enhances the delivery of the drug to a desired site.

33. The method of claim 21 wherein the method further comprises administering a neutrophil migration drug to the patient.

34. The method of claim 33 wherein the neutrophil migration drug is PD98059.

35. The method of claim 33 wherein the neutrophil migration drug enhances transportation of neutrophils across the barrier of the blood vessel.

36. A method of treating a disease in a patient in need thereof, said method comprising the step of administering a nanoparticle composition to the patient, wherein the administration of the nanoparticle composition reduces one or more symptoms associated with the disease.

37. The method of claim 36 wherein the disease is an inflammatory disease.

38. The method of claim 37 wherein the inflammatory disease is an acute inflammatory disease.

39. The method of claim 37 wherein the inflammatory disease is a chronic inflammatory disease.

40. The method of claim 37 wherein the inflammatory disease is cancer.

41. The method of claim 37 wherein the inflammatory disease is sepsis.

42. The method of claim 37 wherein the inflammatory disease is a lung injury.

43. The method of claim 42 wherein the lung injury is an acute lung injury.

44. The method of claim 42 wherein the lung injury is a chronic lung injury.

45. The method of claim 36 wherein the disease is an infection.

46. The method of claim 45 wherein the infection is a bacterial infection.

47. The method of claim 45 wherein the infection is a viral infection.

48. The method of claim 45 wherein the infection is a fungal infection.

49. The method of claim 36 wherein the administration of the nanoparticle composition comprises transportation across a blood vessel barrier in the patient.

50. The method of claim 49 wherein the transporting across the blood vessel is via a transcellular pathway.

51. The method of claim 49 wherein the transporting across the blood vessel is via a neutrophil transmigration pathway.

52. The method of claim 51 wherein the neutrophil is a polymorphonuclear neutrophil.

53. The method of claim 51 wherein the neutrophil is an activated neutrophil.

54. The method of claim 51 wherein the neutrophil comprises a Fcγ receptor.

55. The method of claim 51 wherein the nanoparticle composition is internalized into the neutrophil.

56. The method of claim 51 wherein the barrier of the blood vessel is an endothelial vessel barrier.

57. The method of claim 51 wherein the blood vessel is a lung blood vessel.

58. The method of claim 49 wherein the transporting across the blood vessel enhances the delivery of the drug to a desired site.

59. The method of claim 36 wherein the method further comprises administering a neutrophil migration drug to the patient.

60. The method of claim 59 wherein the neutrophil migration drug is PD98059.

61. The method of claim 59 wherein the neutrophil migration drug enhances transportation of neutrophils across the barrier of the blood vessel.

62. The method of claim 36 wherein the administration is a parenteral administration.

63. The method of claim 62 wherein the parenteral administration is an intravenous administration.

64. The method of claim 36 wherein the drug is an antibiotic.

65. The method of claim 36 wherein the drug is an anti-inflammatory drug.

66. The method of claim 36 wherein the drug is an anticancer drug.

67. The method of claim 36 wherein the drug is an NF-κB inhibitor.

68. The method of claim 67 wherein the NF-κB inhibitor is TPCA-1.

69. The method of claim 36 wherein the drug is cefoperazone acid.

70. The method of claim 36 wherein the albumin protein is a bovine serum albumin (BSA) protein.

71. The method of claim 70 wherein the BSA protein is a denatured BSA protein.

72. The method of claim 36 wherein the nanoparticle composition is conjugated to a polyethylene glycol moiety.

73. The method of claim 36 wherein the nanoparticle composition has a diameter between about 100 nm to about 150 nm.

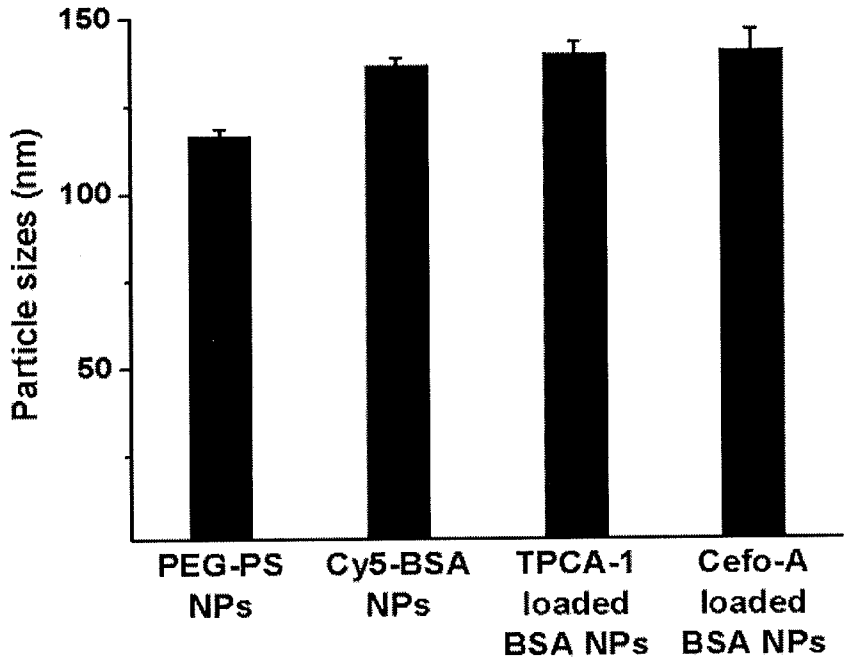
150
100
50
Particle sizes (nm)
PEG-PS NPs
Cy5-BSA NPs
TPCA-1 loaded BSA NPs
Cefo-A loaded BSA NPs

FIG. 1A

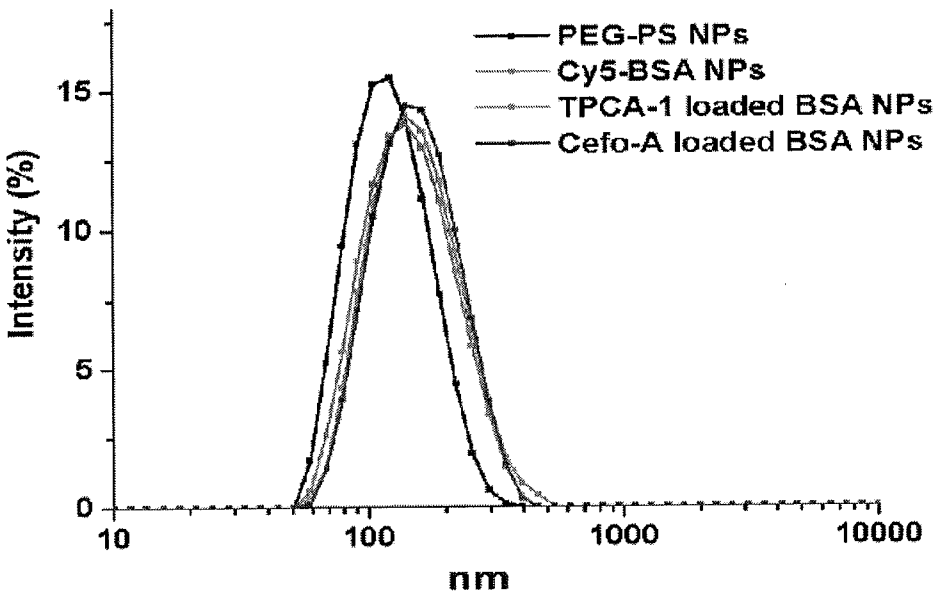
PEG-PS NPs
Cy5-BSA NPs
TPCA-1 loaded BSA NPs
Cefo-A loaded BSA NPs
15
10
5
0
Intensity (%)
10
100
1000
10000
nm

FIG. 1B

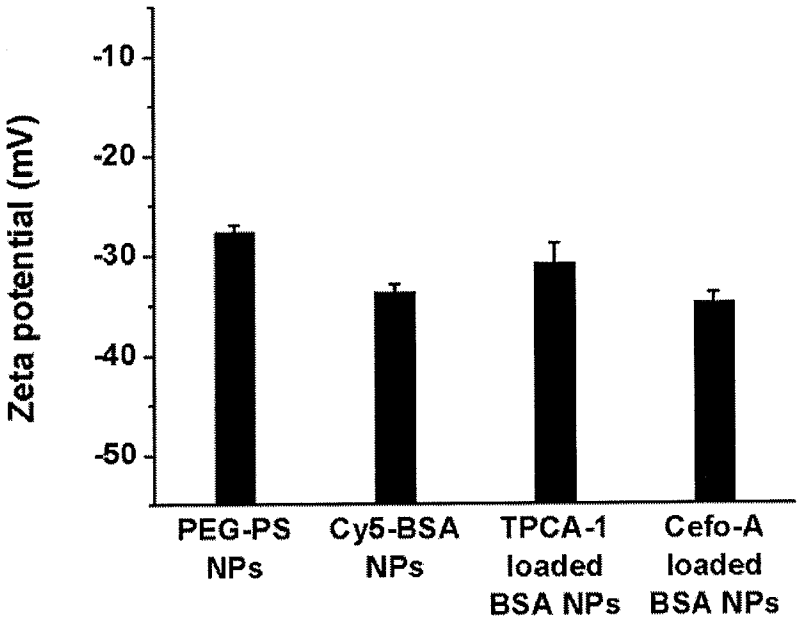
Zeta potential (mV)
-10
-20
-30
-40
-50
PEG-PS NPs
Cy5-BSA NPs
TPCA-1 loaded BSA NPs
Cefo-A loaded BSA NPs

FIG. 1C

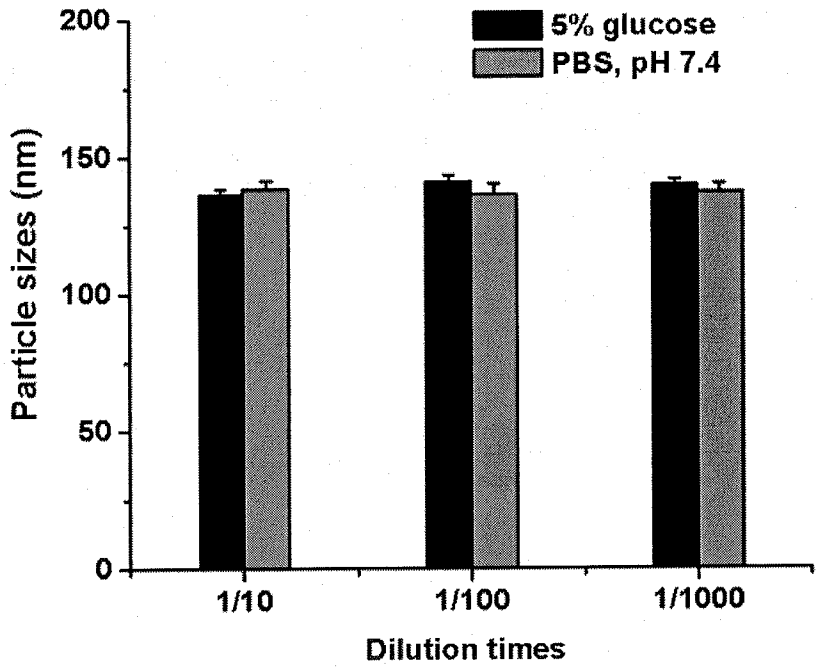
5% glucose
PBS, pH 7.4
Particle sizes (nm)
200
150
100
50
0
1/10
1/100
1/1000
Dilution times

FIG. 1D

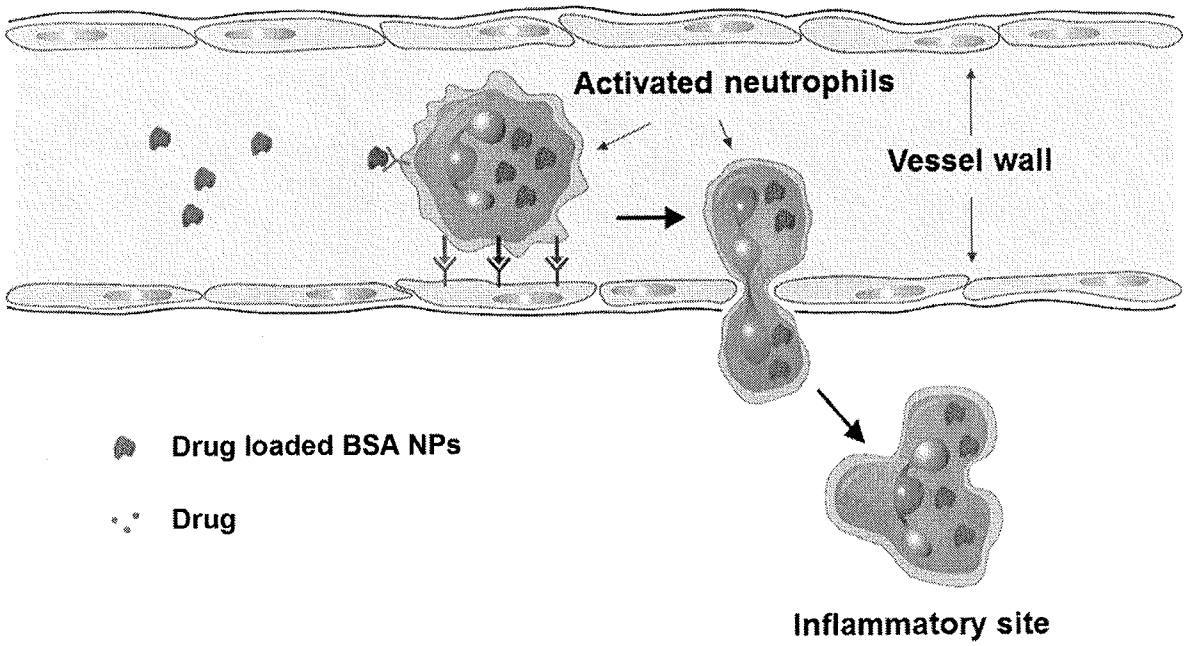
Activated neutrophils
Vessel wall
Drug loaded BSA NPs
Drug
Inflammatory site

FIG. 2A

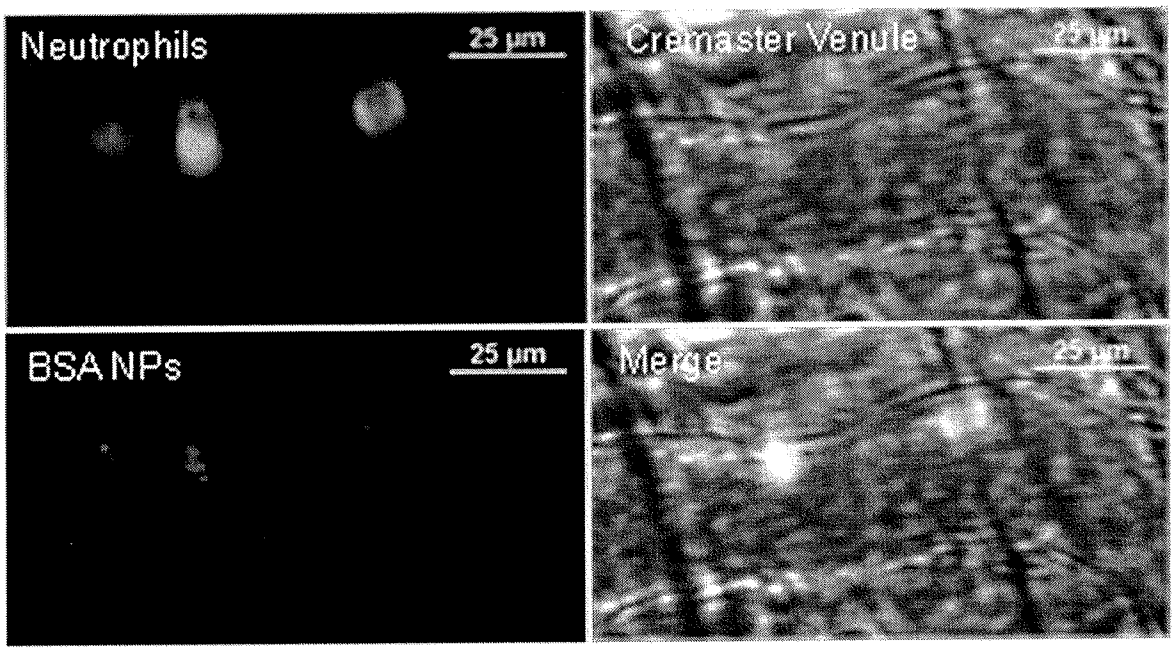
Neutrophils
25 μm
Cremaster Venule
25 μm
BSA NPs
25 μm
Merge
25 μm

FIG. 2B

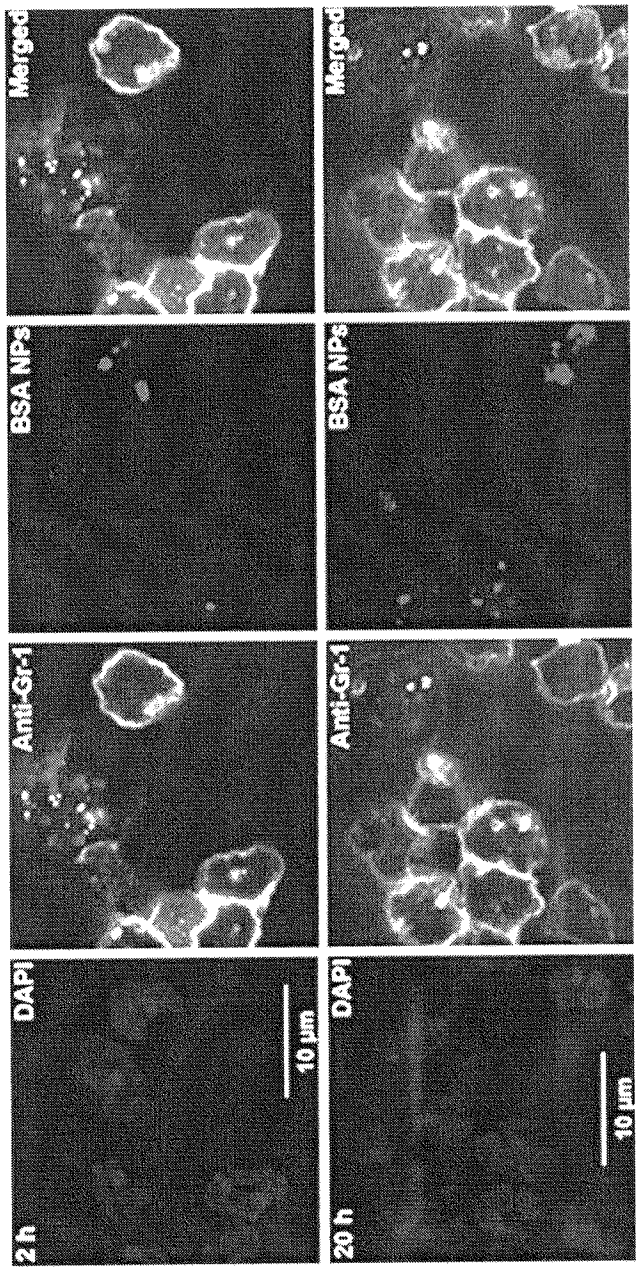

FIG. 2C FIG. 2D

FIG. 2C & 2D

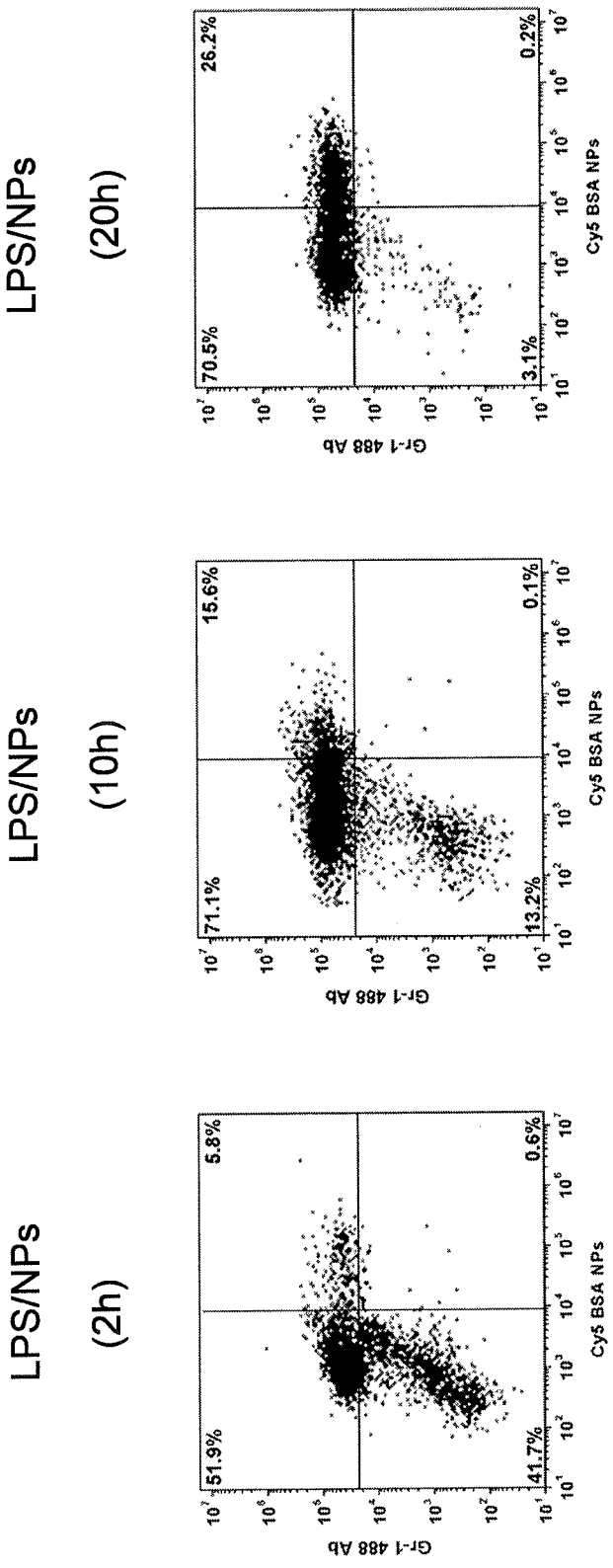
LPS/NPs (2h)
LPS/NPs (10h)
LPS/NPs (20h)
Gr-1 488 Ab
Cy5 BSA NPs
5.8%
0.6%
51.9%
41.7%
15.6%
0.1%
71.1%
13.2%
26.2%
0.2%
70.5%
3.1%

FIG. 2E

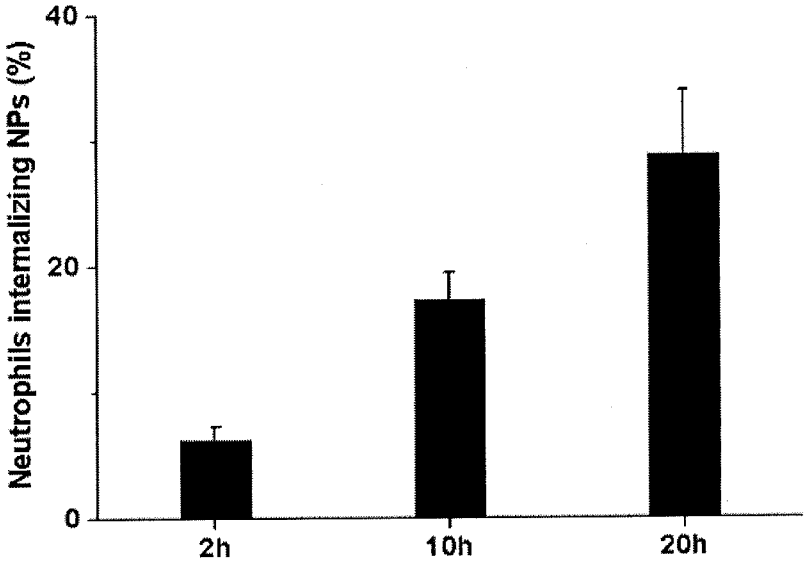
Neutrophils internalizing NPs (%)
40
20
0
2h
10h
20h

FIG. 2F

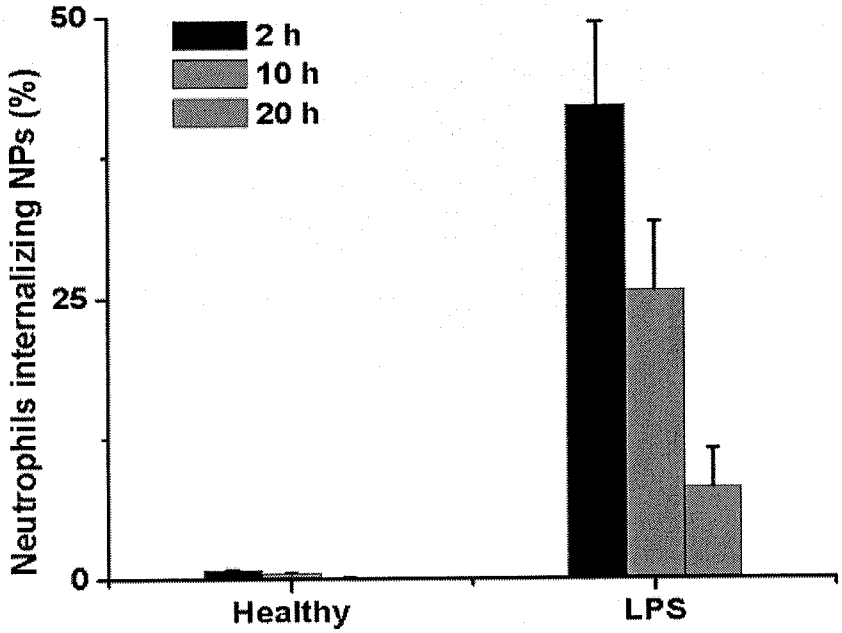
Neutrophils internalizing NPs (%)
50
25
0
2 h
10 h
20 h
Healthy
LPS

FIG. 2G

A

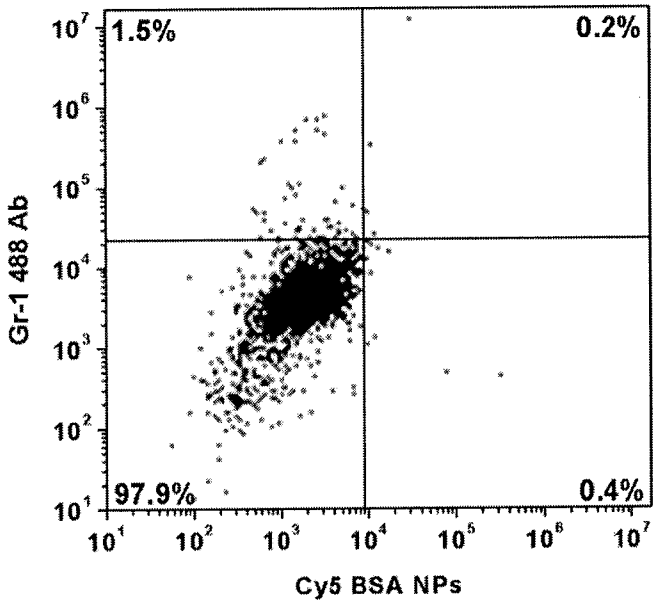

FIG. 3A

B

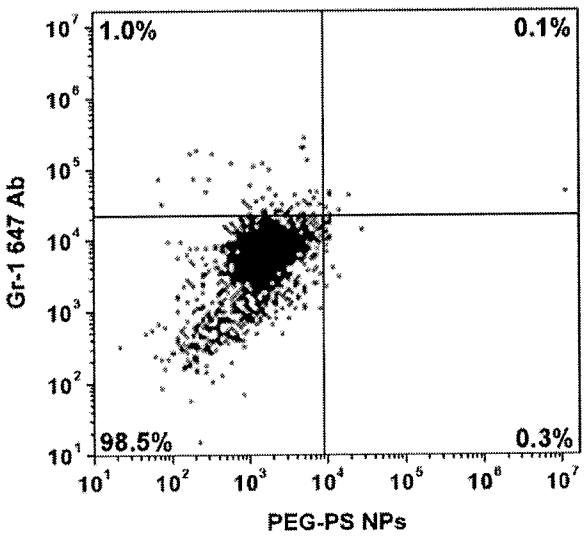

FIG. 3B

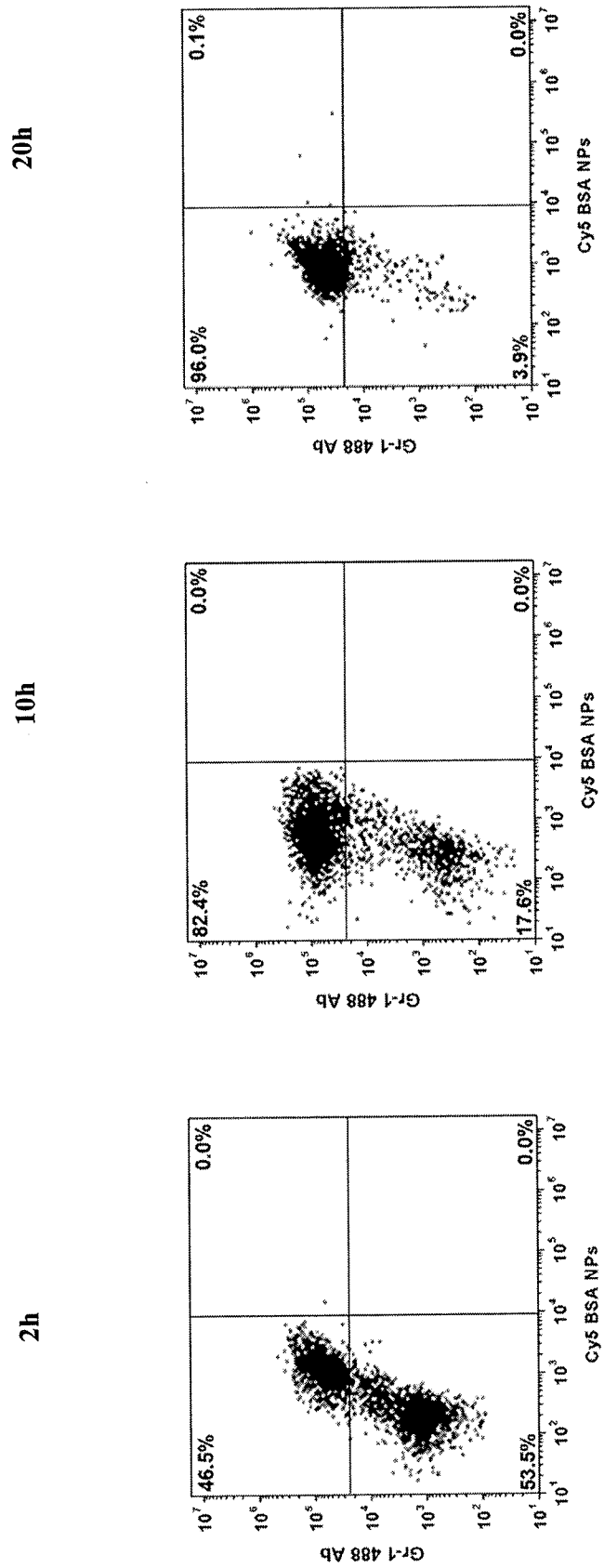
2h
10h
20h
0.1%
0.0%
96.0%
3.9%
0.0%
0.0%
82.4%
17.6%
0.0%
0.0%
46.5%
53.5%
Gr-1 488 Ab
Cy5 BSA NPs

FIG. 4

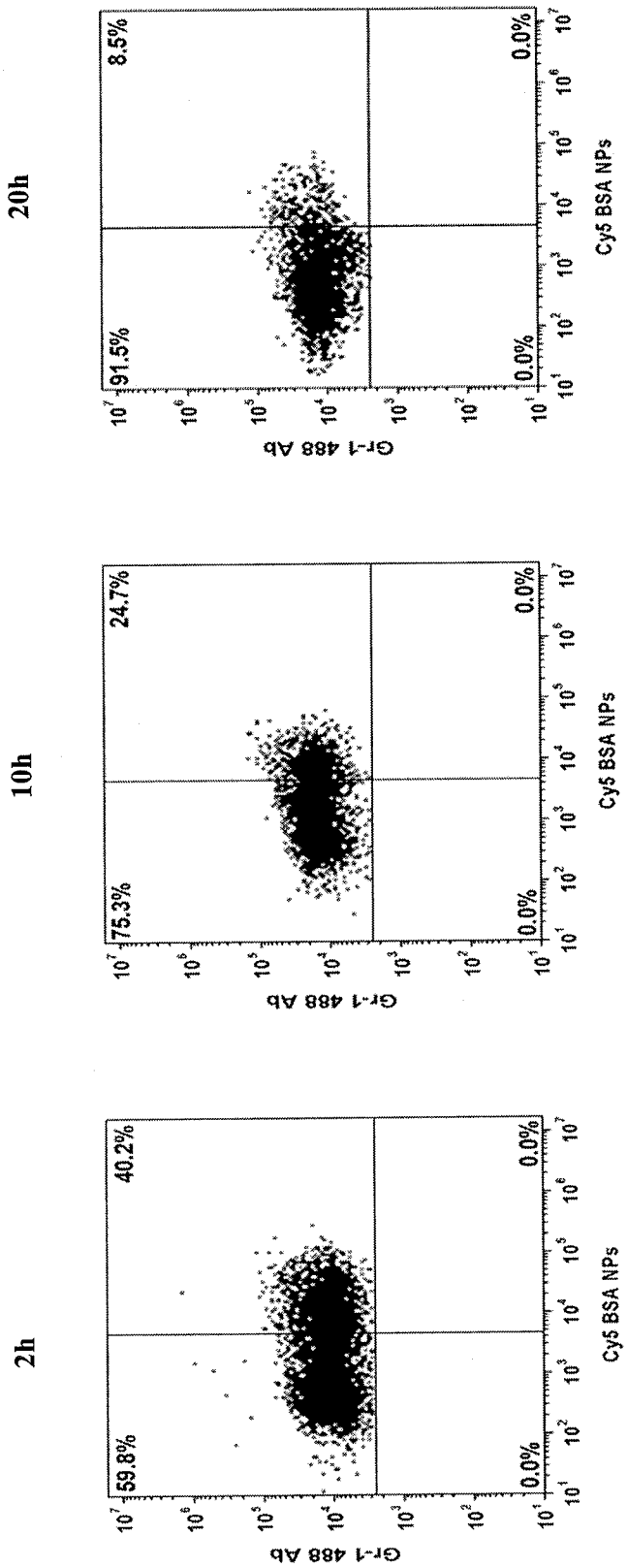

FIG. 5A: LPS, Cy5-BSA NPs

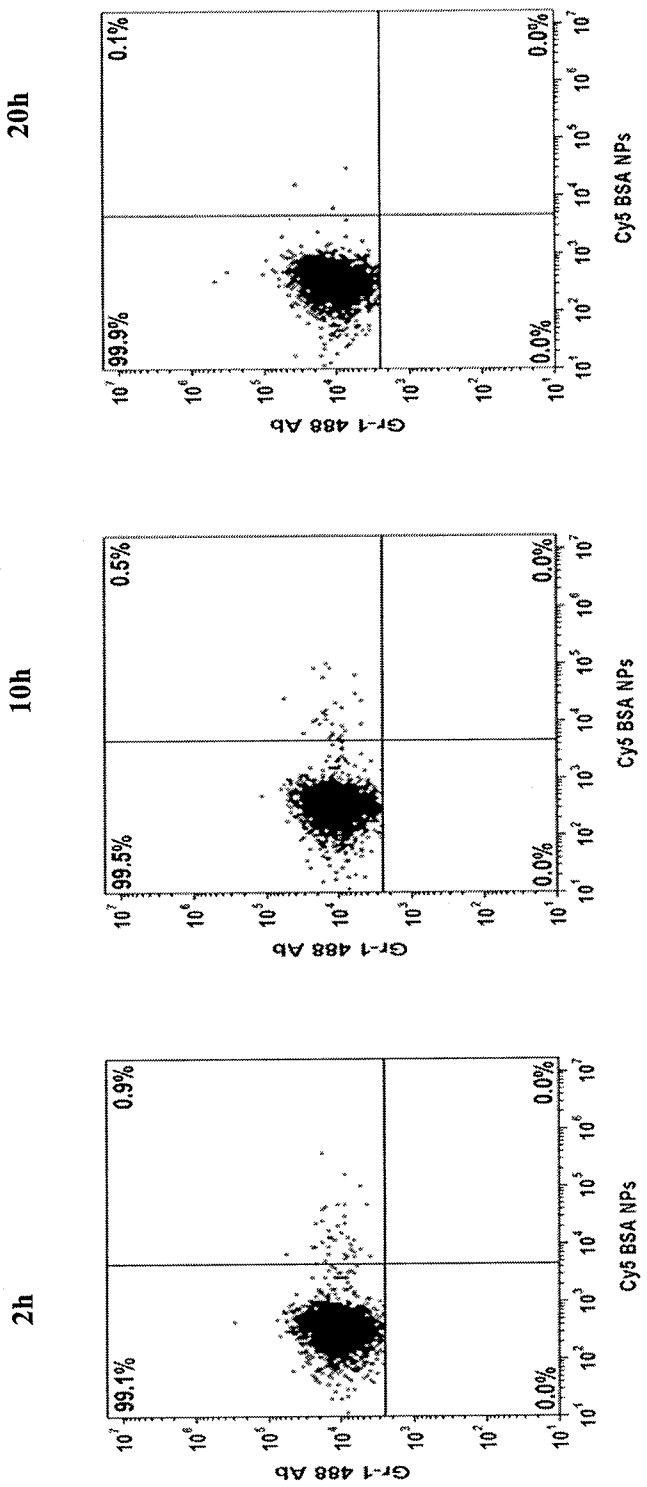

FIG. 5B: Healthy, Cy5-BSA NPs

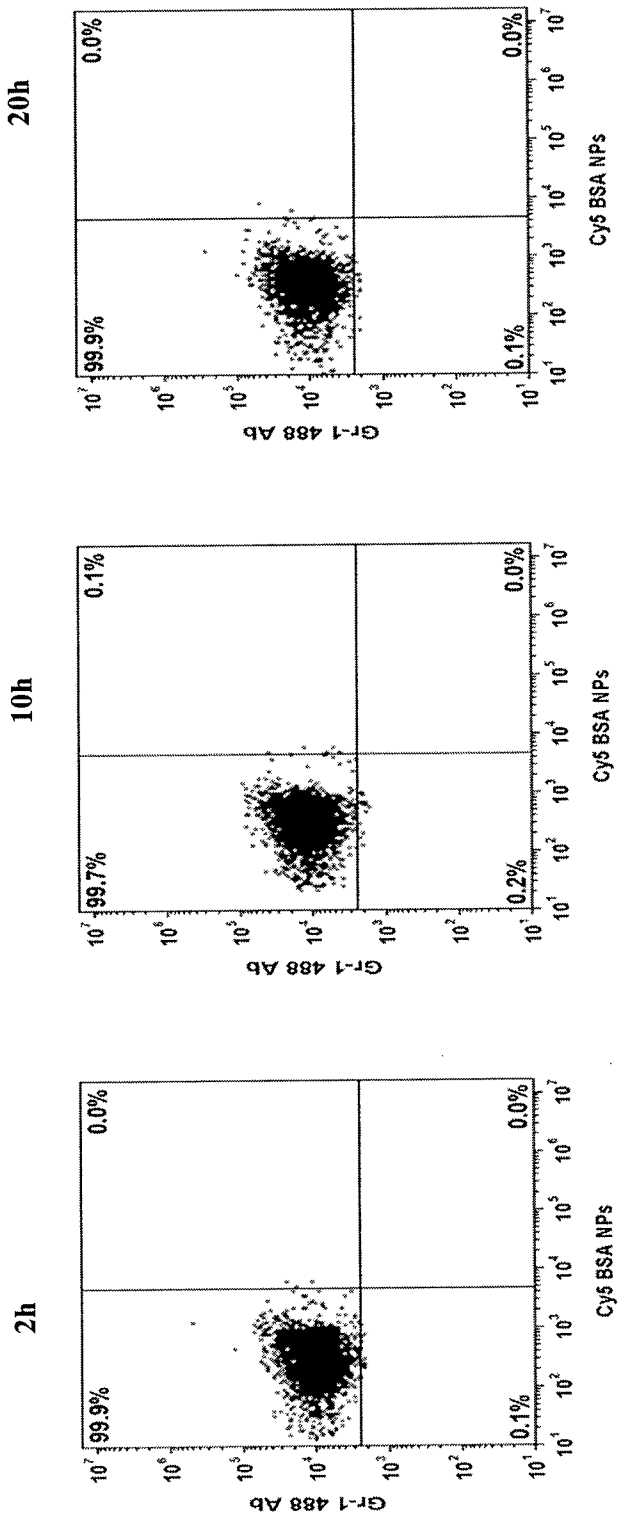

FIG. 5C: LPS, W/O Cy5-BSA NPs

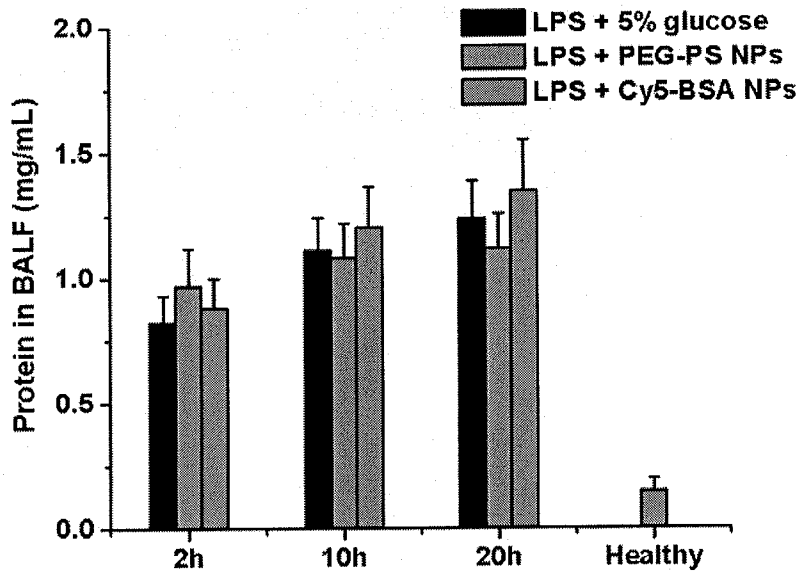
LPS + 5% glucose
LPS + PEG-PS NPs
LPS + Cy5-BSA NPs
Protein in BALF (mg/mL)
2.0
1.5
1.0
0.5
0.0
2h
10h
20h
Healthy

FIG. 6A

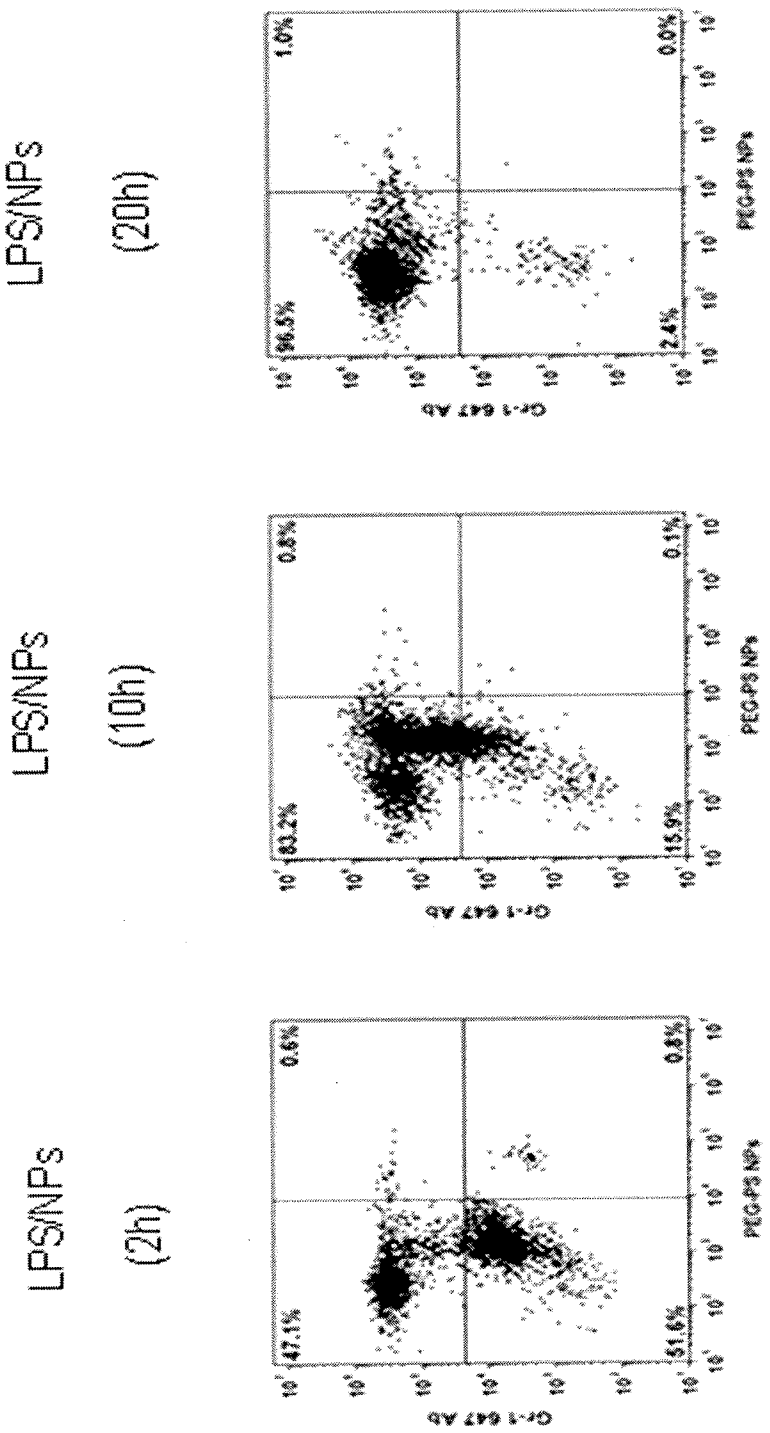
LPS/NPs (20h)
LPS/NPs (10h)
LPS/NPs (2h)
1.0%
0.0%
96.5%
2.4%
0.8%
0.1%
83.2%
15.9%
0.6%
0.8%
47.1%
51.6%
Cy-1 647 Ab
PEG-PS NPs

FIG. 6B

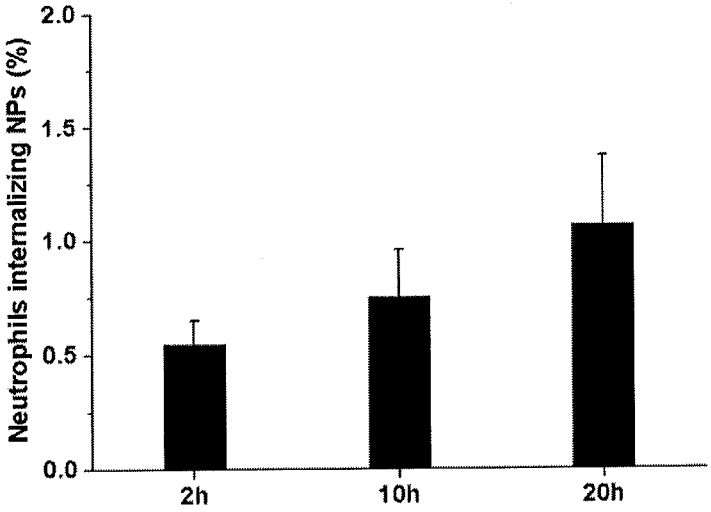
Neutrophils internalizing NPs (%)
2.0
1.5
1.0
0.5
0.0
2h
10h
20h

FIG. 6C

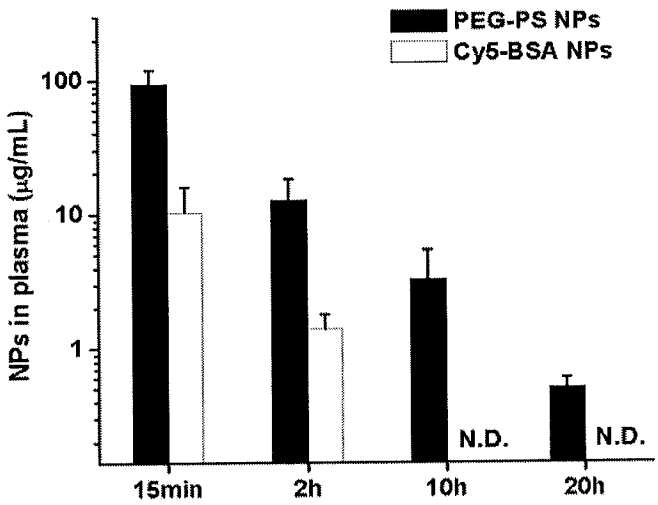
PEG-PS NPs
Cy5-BSA NPs
NPs in plasma (μg/mL)
100
10
1
N.D.
N.D.
15min
2h
10h
20h

FIG. 6D

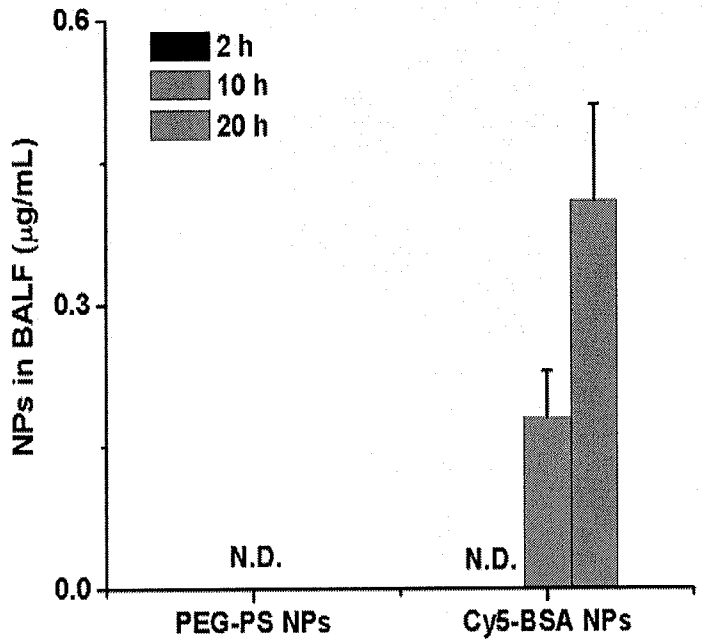

FIG. 6E

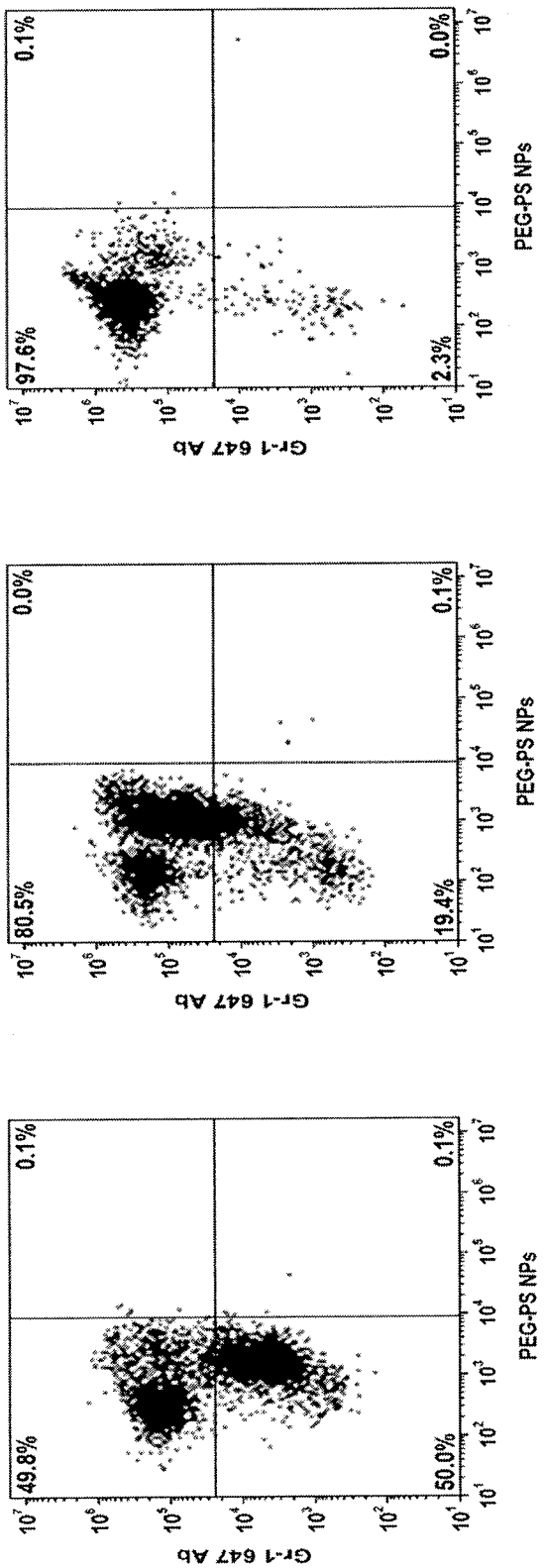
0.1%
0.0%
97.6%
2.3%
Gr-1 647 Ab
PEG-PS NPs
0.0%
0.1%
80.5%
19.4%
Gr-1 647 Ab
PEG-PS NPs
0.1%
0.1%
49.8%
50.0%
Gr-1 647 Ab
PEG-PS NPs

FIG. 7

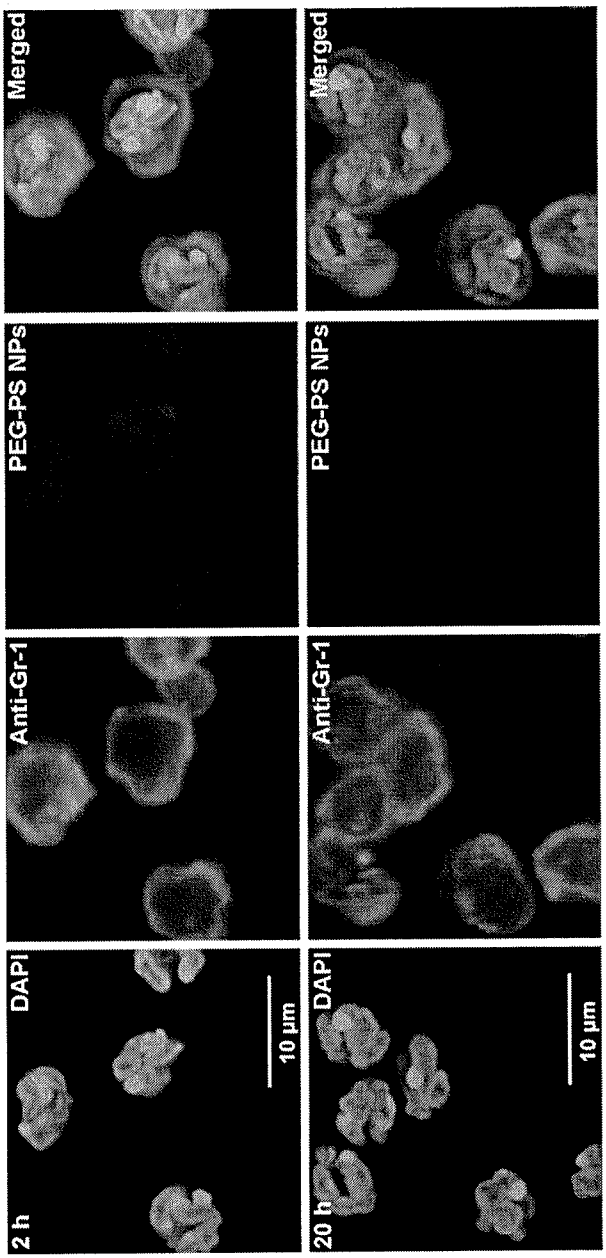
2 h
DAPI
10 µm
Anti-Gr-1
PEG-PS NPs
Merged
20 h
DAPI
10 µm
Anti-Gr-1
PEG-PS NPs
Merged

FIG. 8A

FIG. 8B

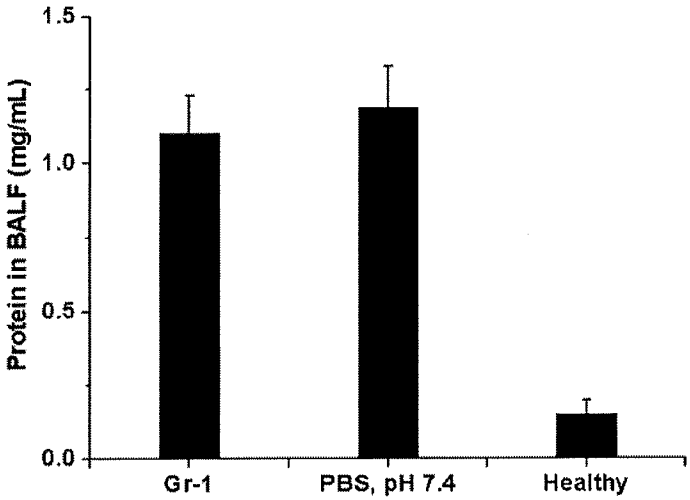
Protein in BALF (mg/mL)
1.5
1.0
0.5
0.0
Gr-1
PBS, pH 7.4
Healthy

FIG. 9A

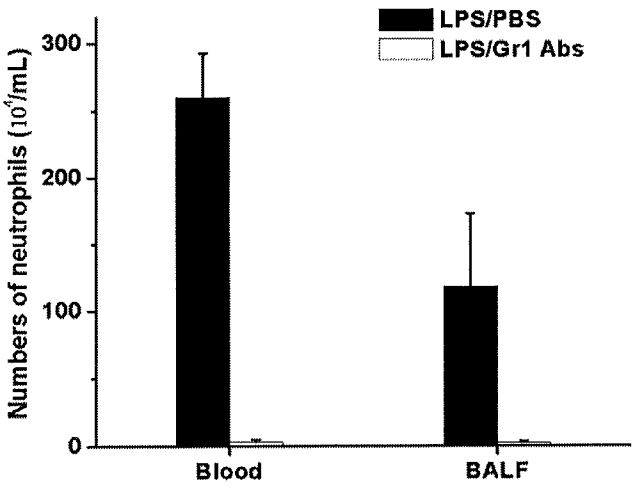
LPS/PBS
LPS/Gr1 Abs
Numbers of neutrophils (10^4/mL)
300
200
100
0
Blood
BALF

FIG. 9B

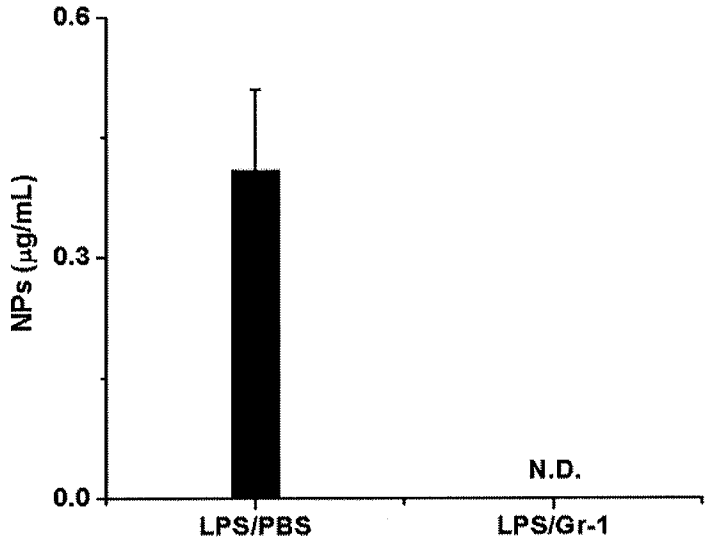

FIG. 9C

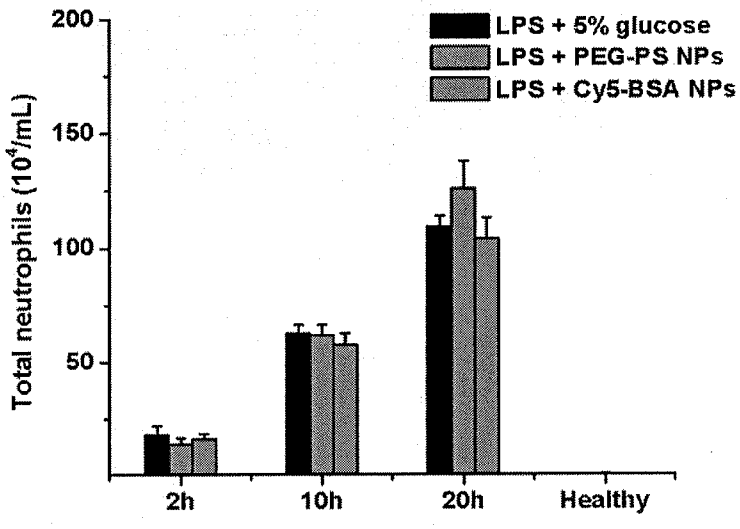

FIG. 10A

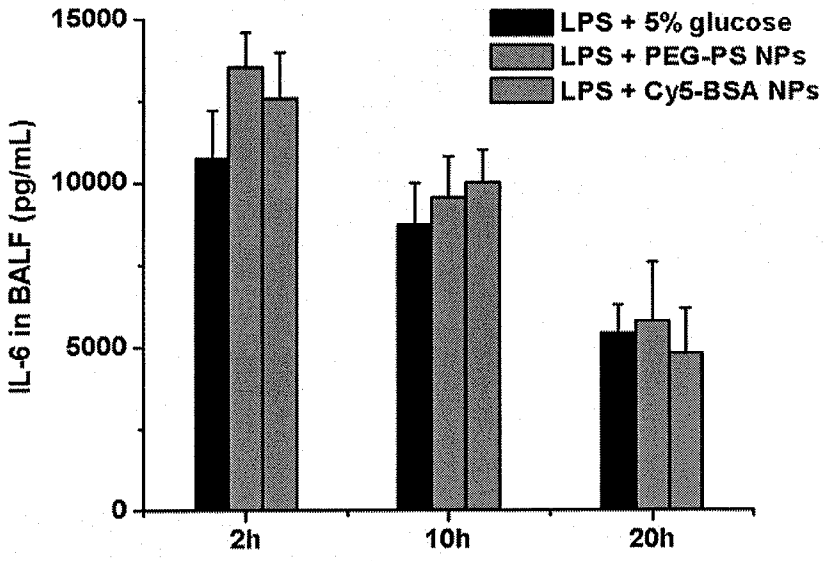

FIG. 10B

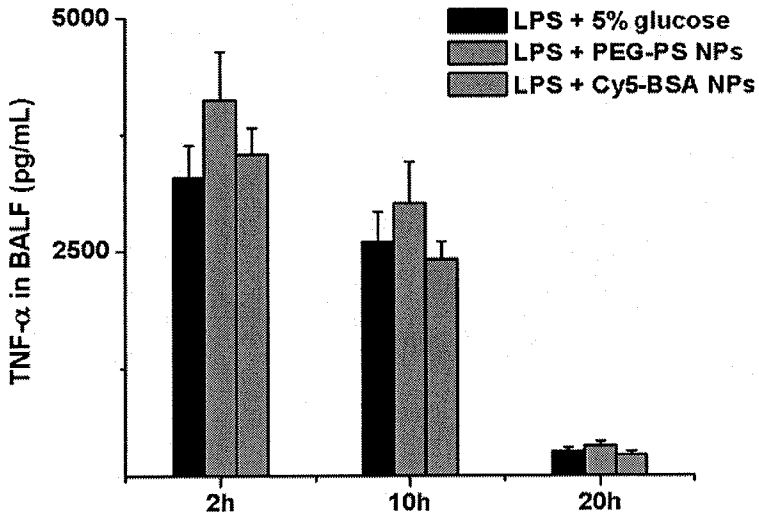

FIG. 10C

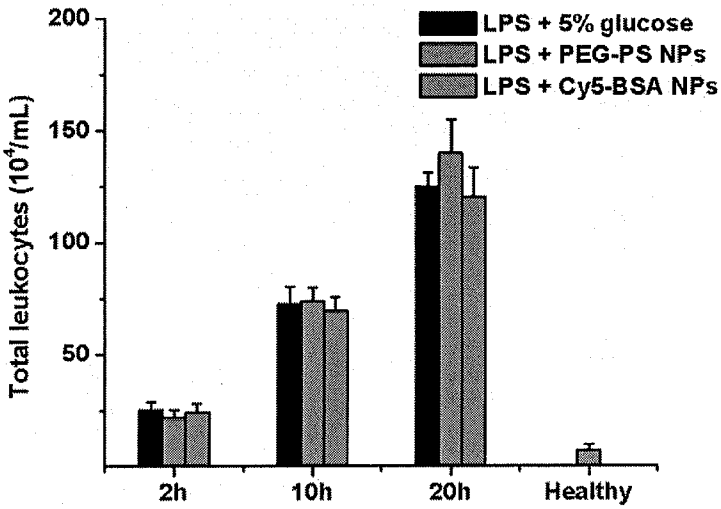

FIG. 11

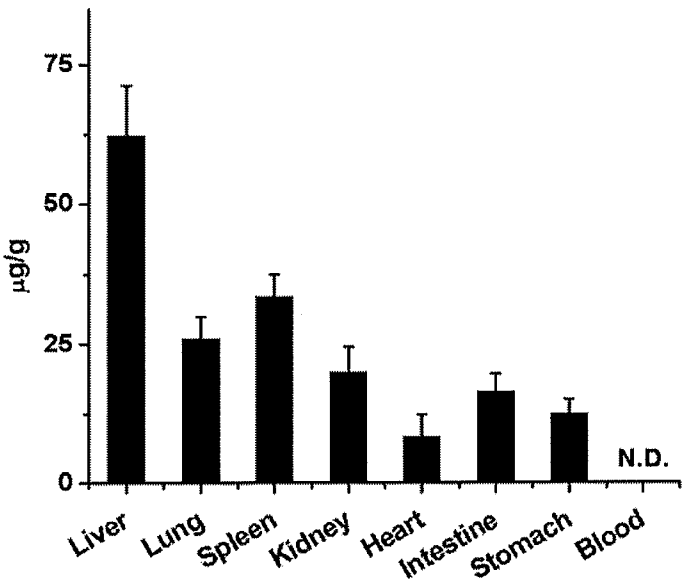

FIG. 12A

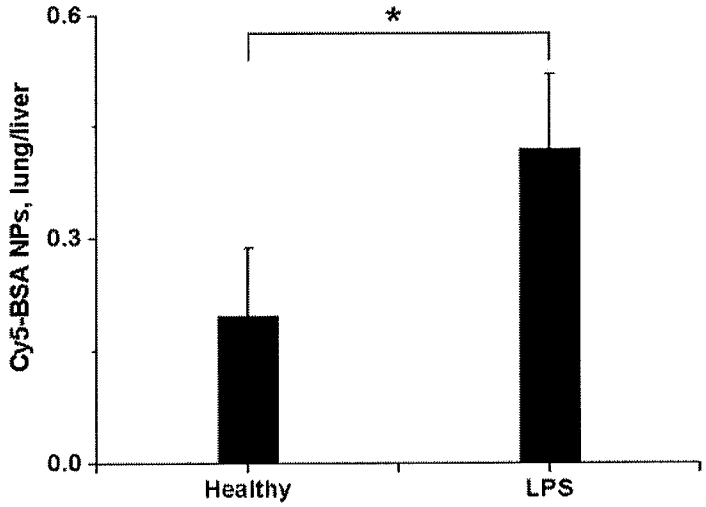

FIG. 12B

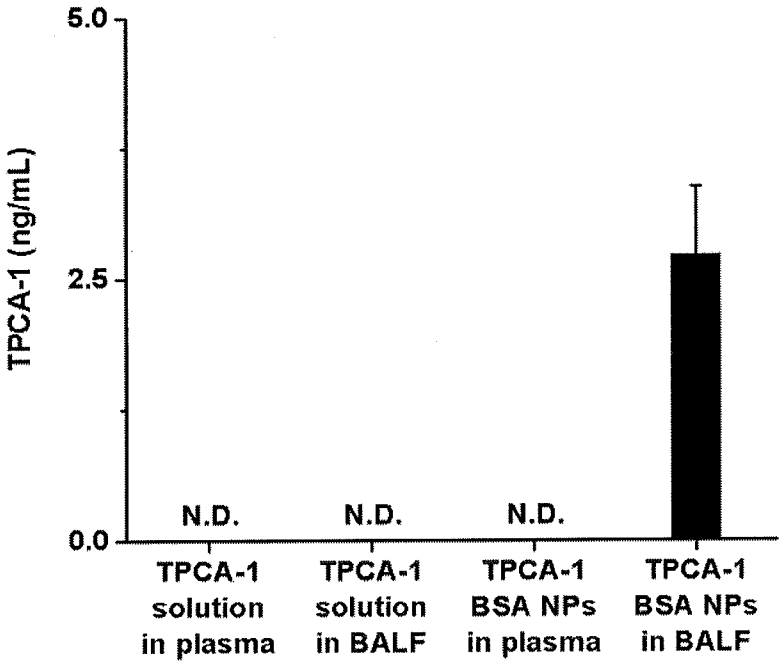

FIG. 13A

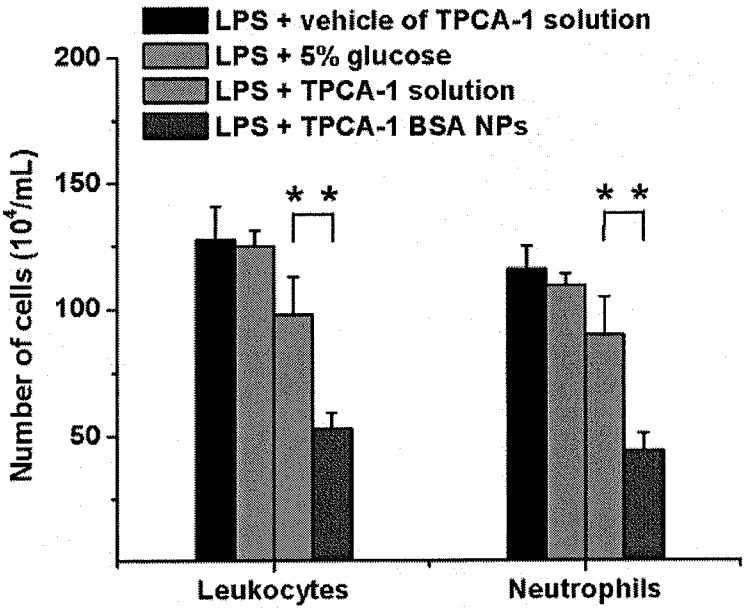

FIG. 13B

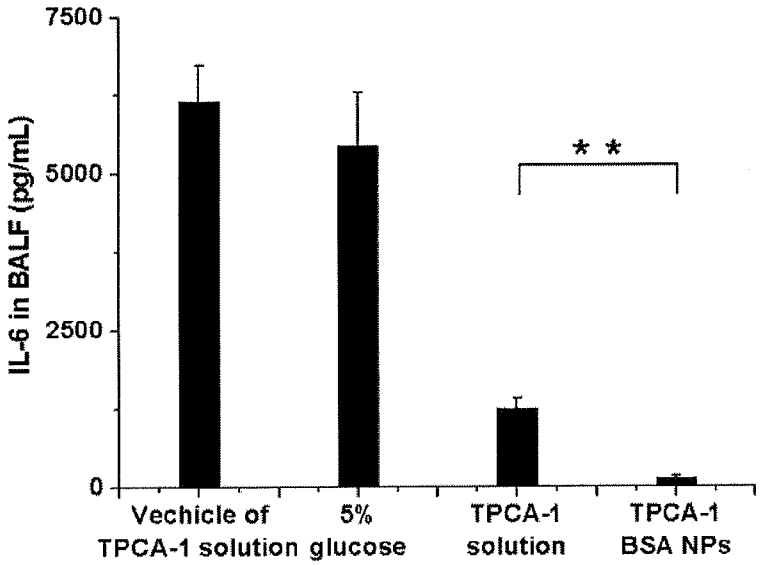

FIG. 13C

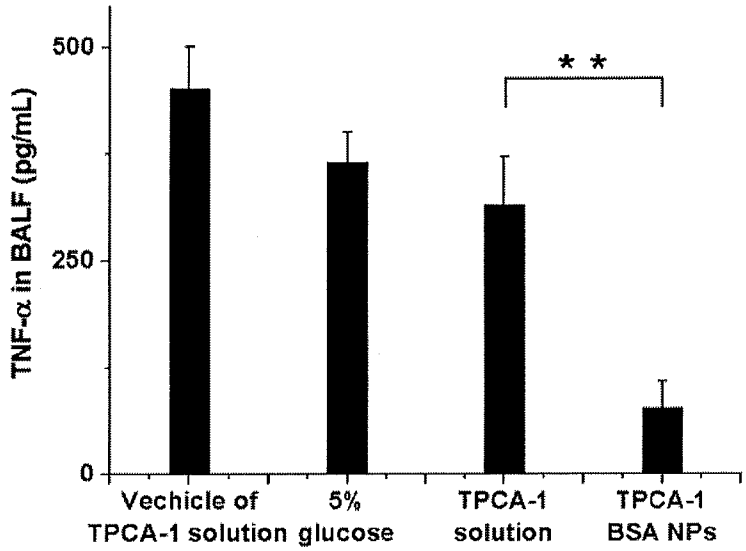

FIG. 13D

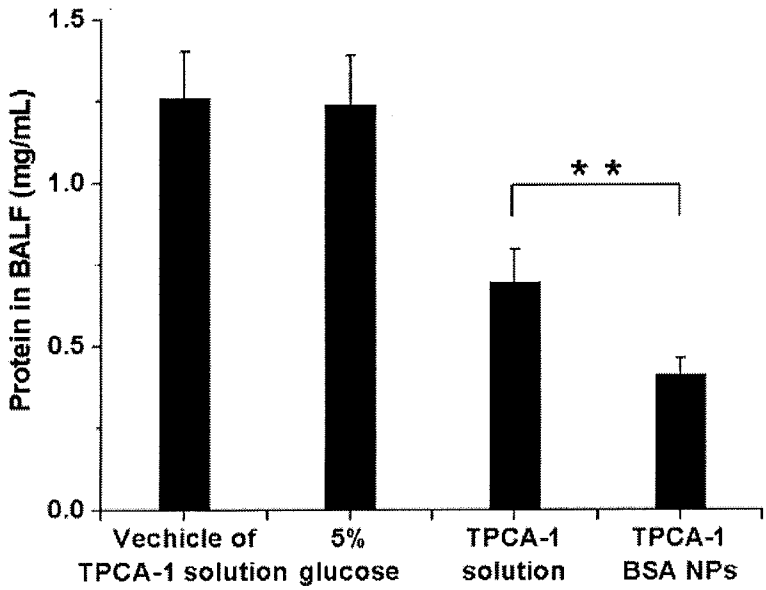

FIG. 13E

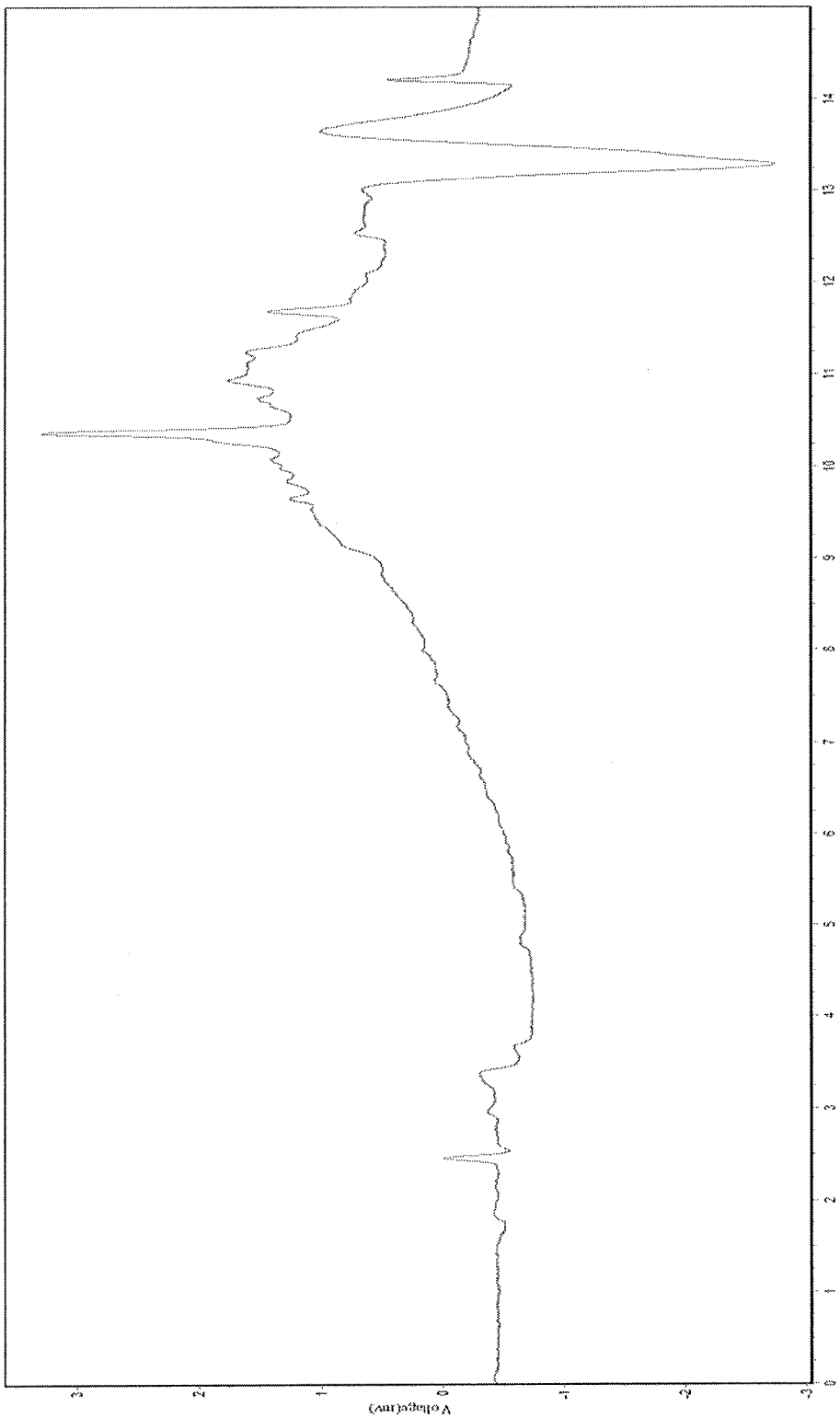

FIG. 14A

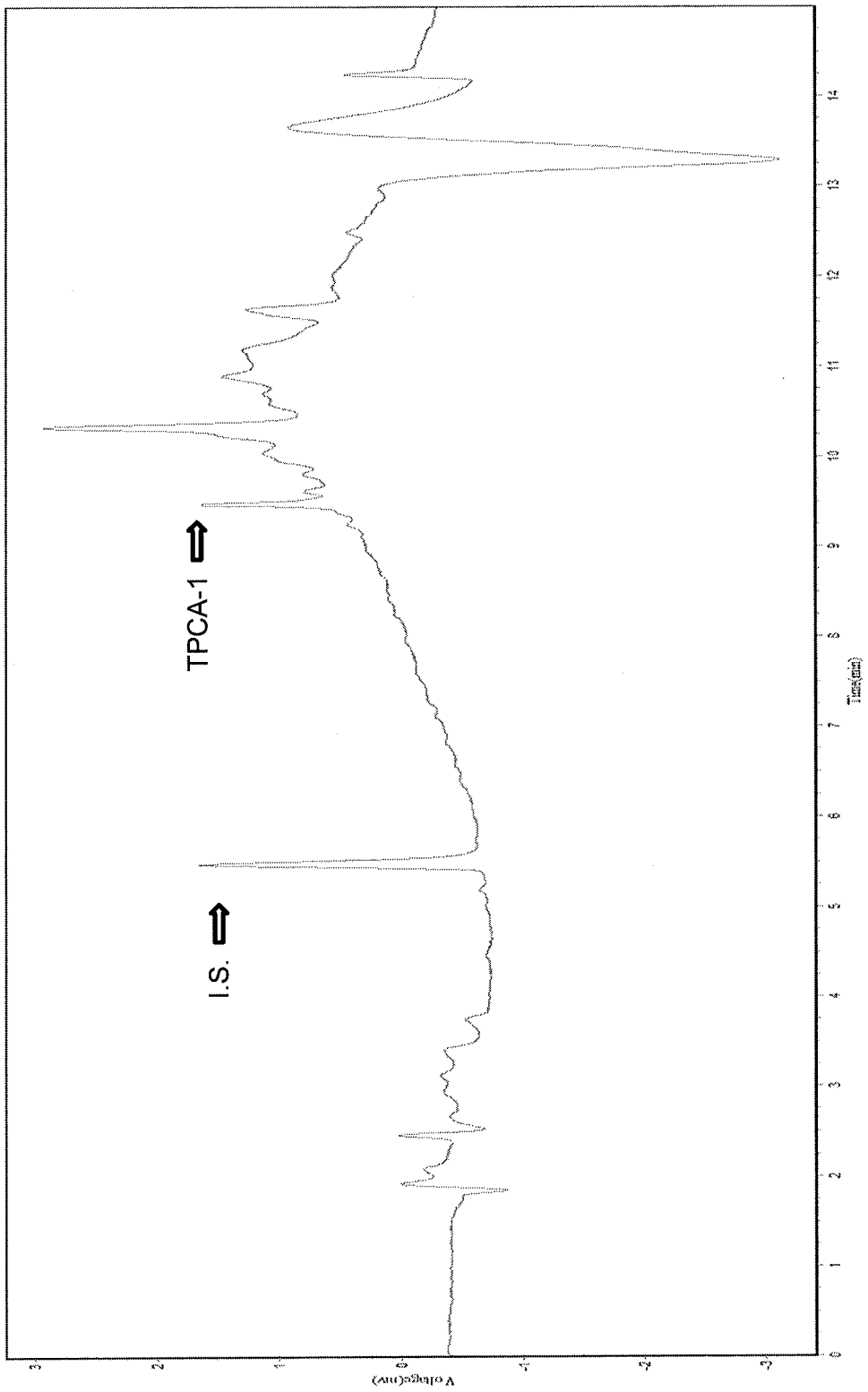

FIG. 14B

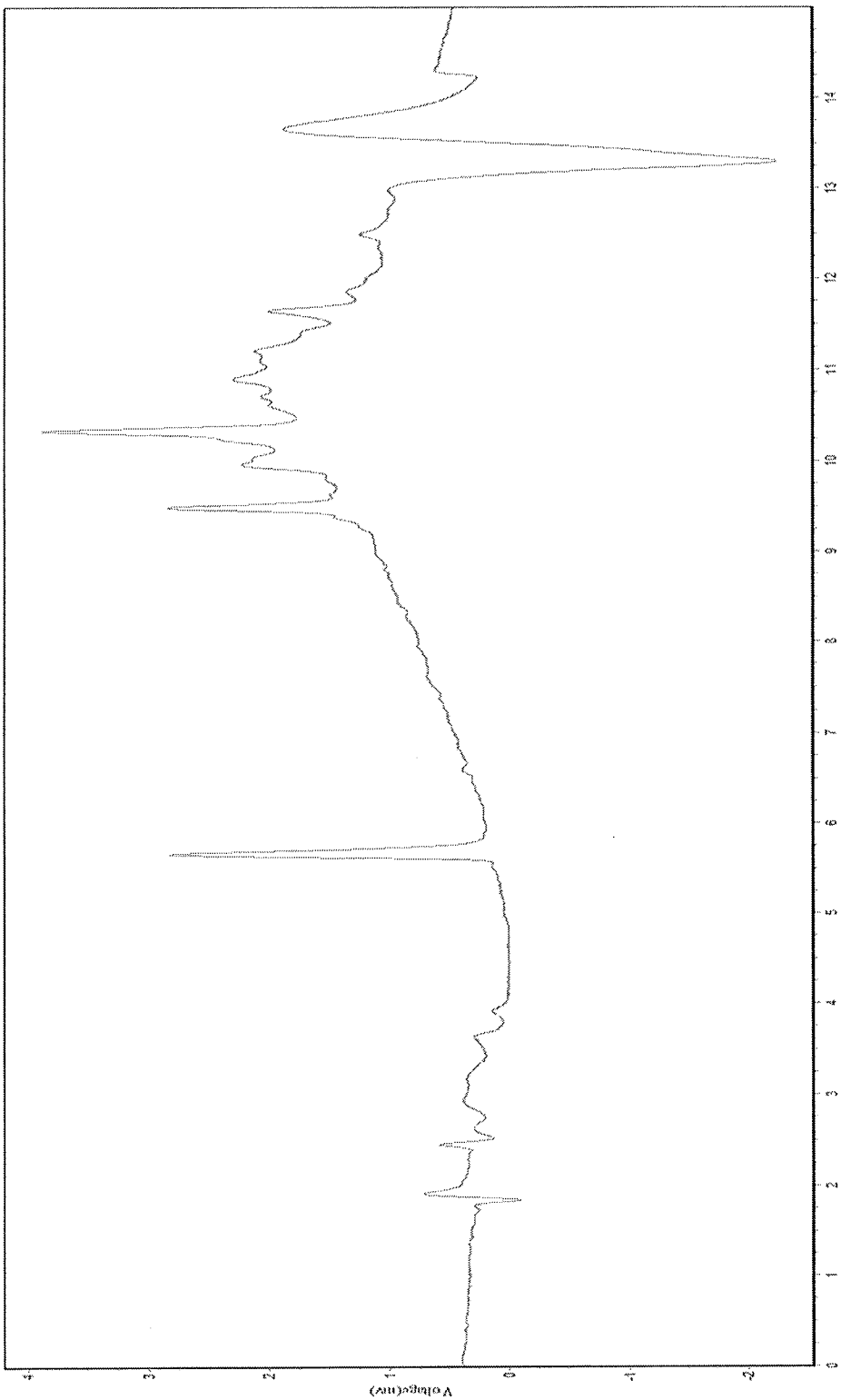

FIG. 14C

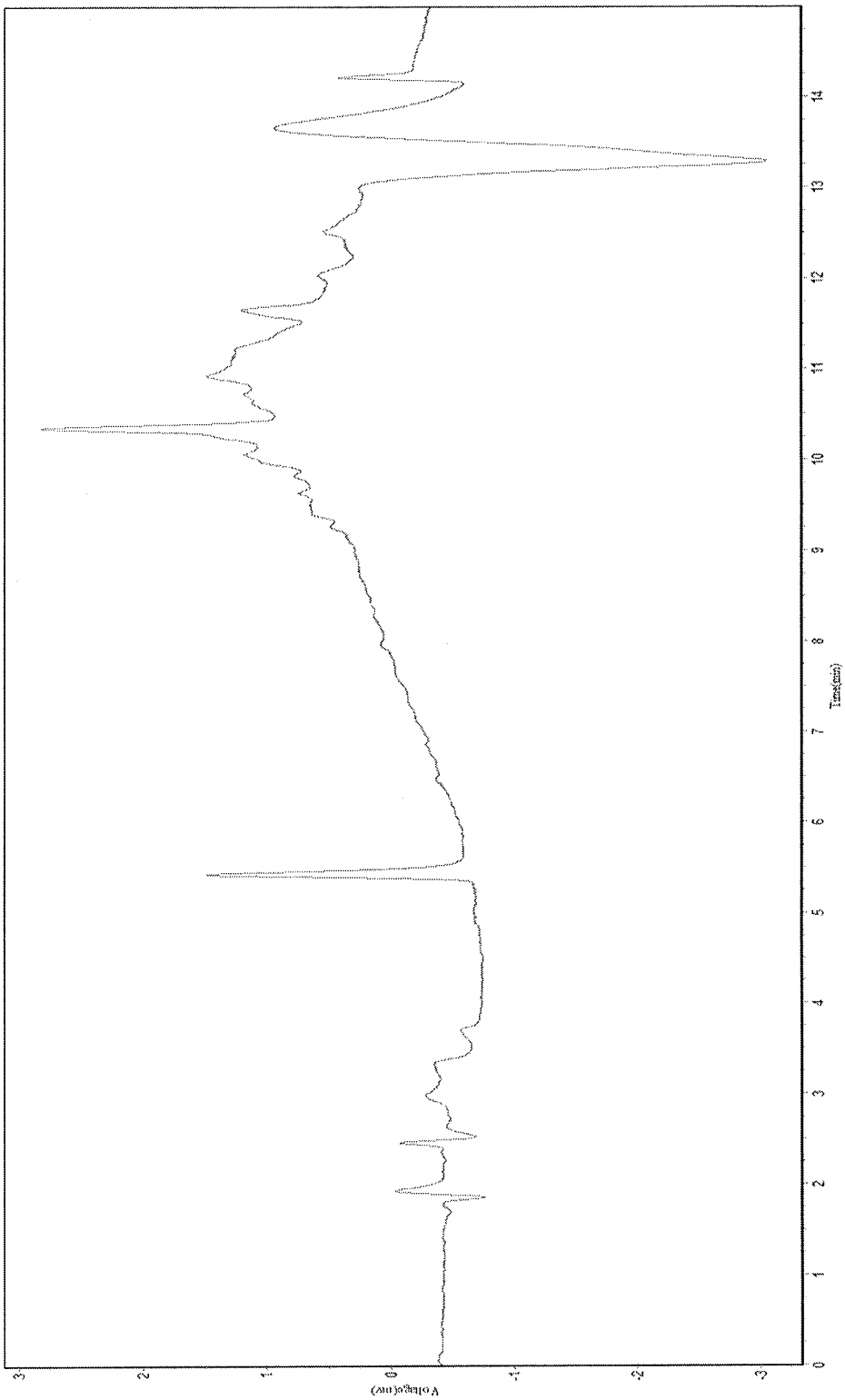

FIG. 14D

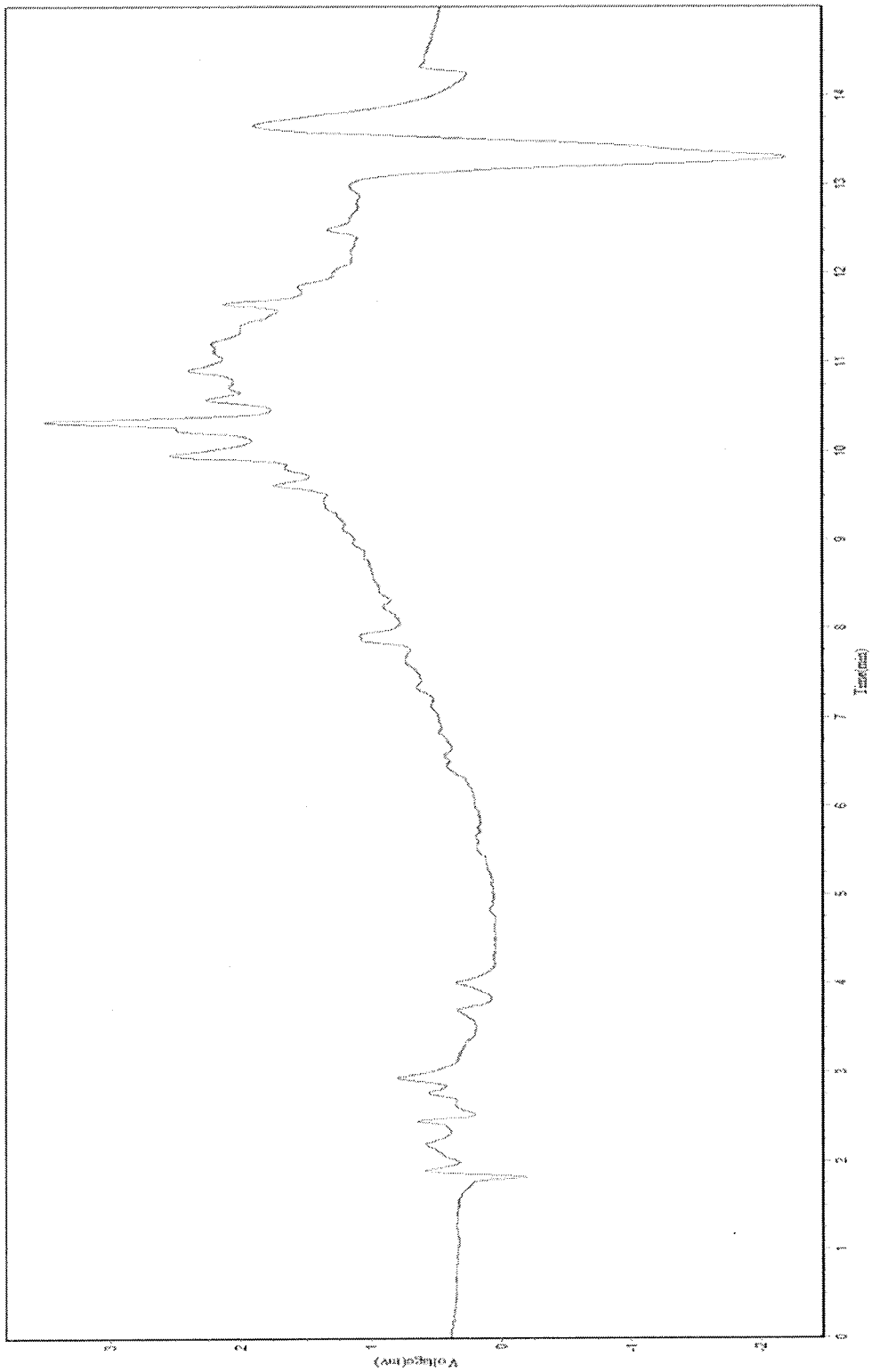

FIG. 15A

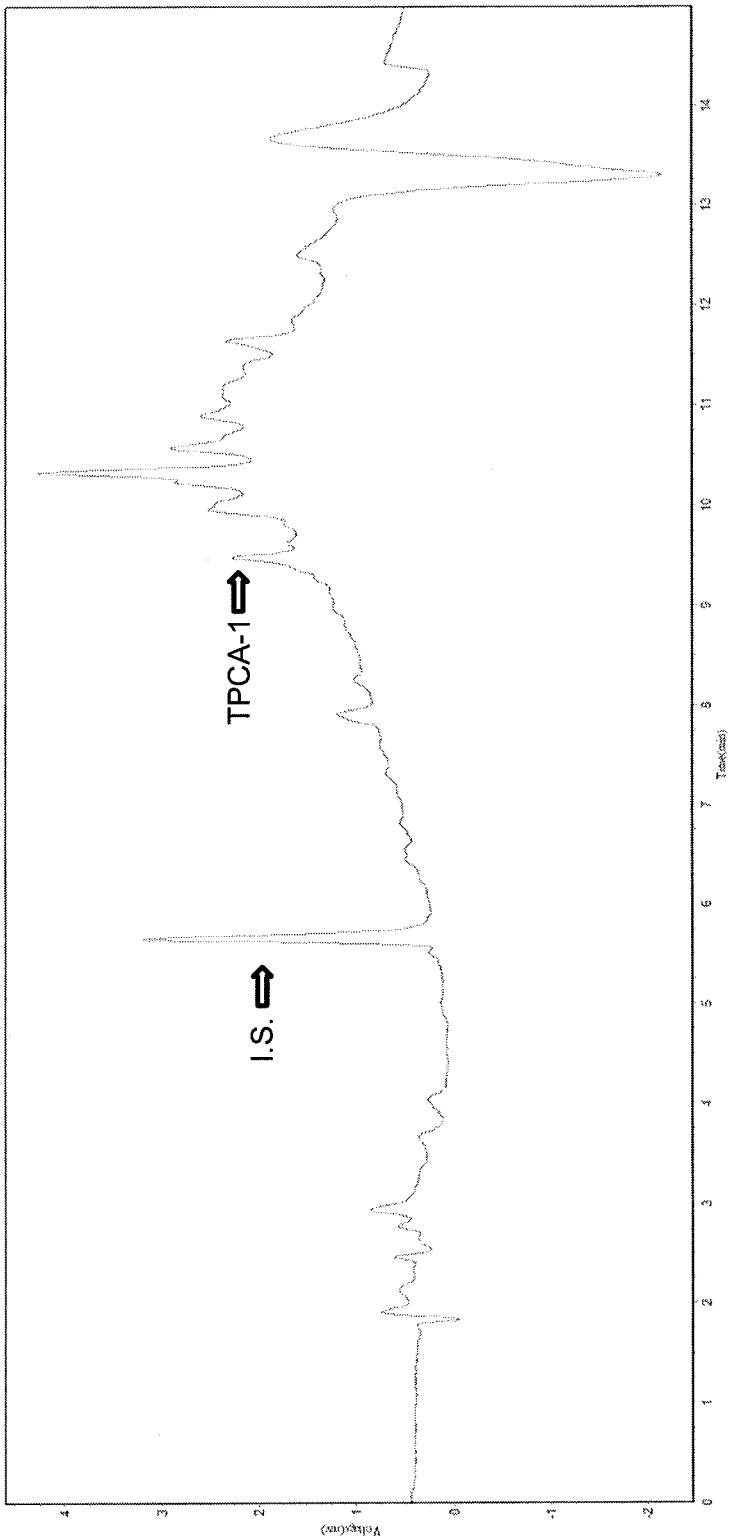

FIG. 15A

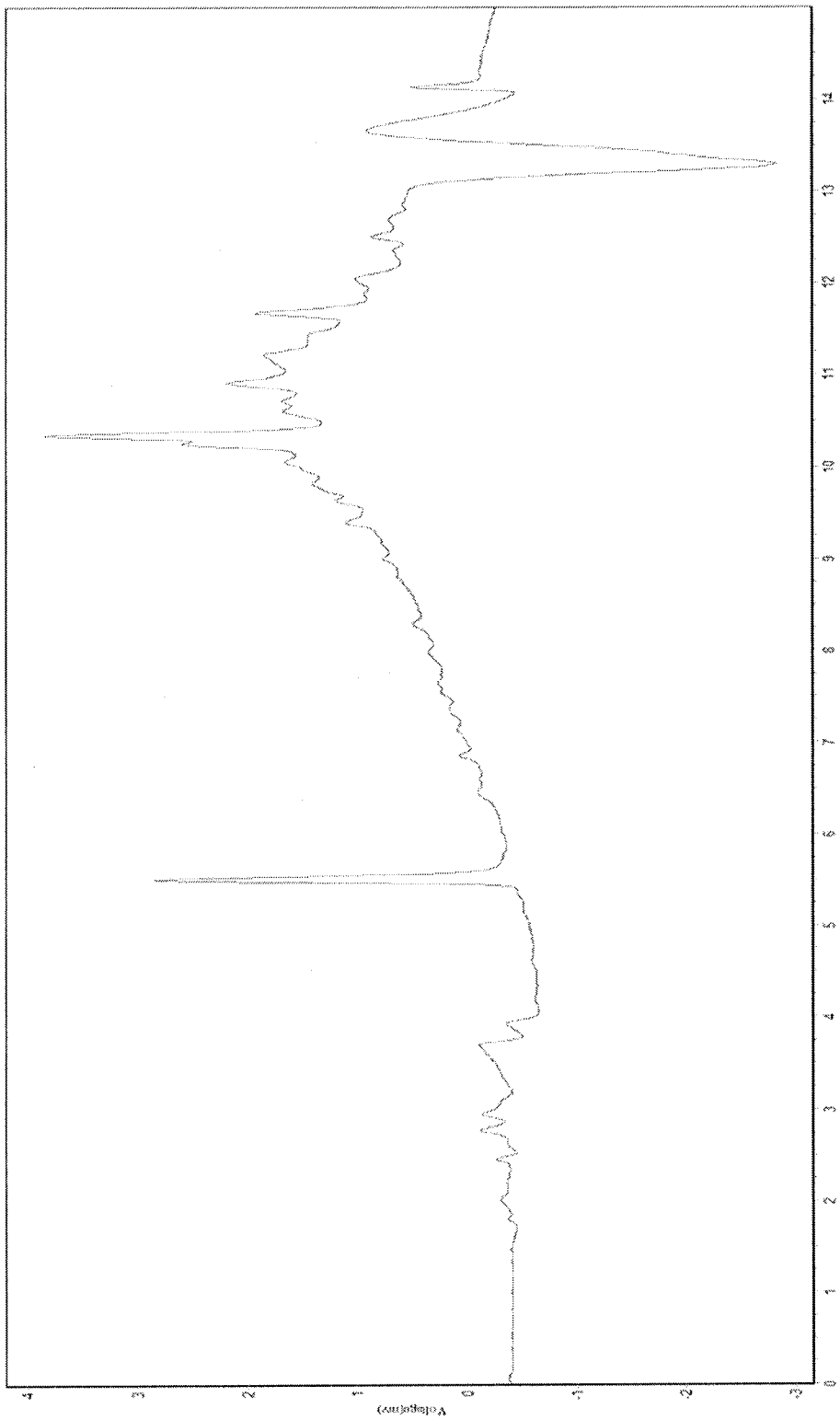

FIG. 15C

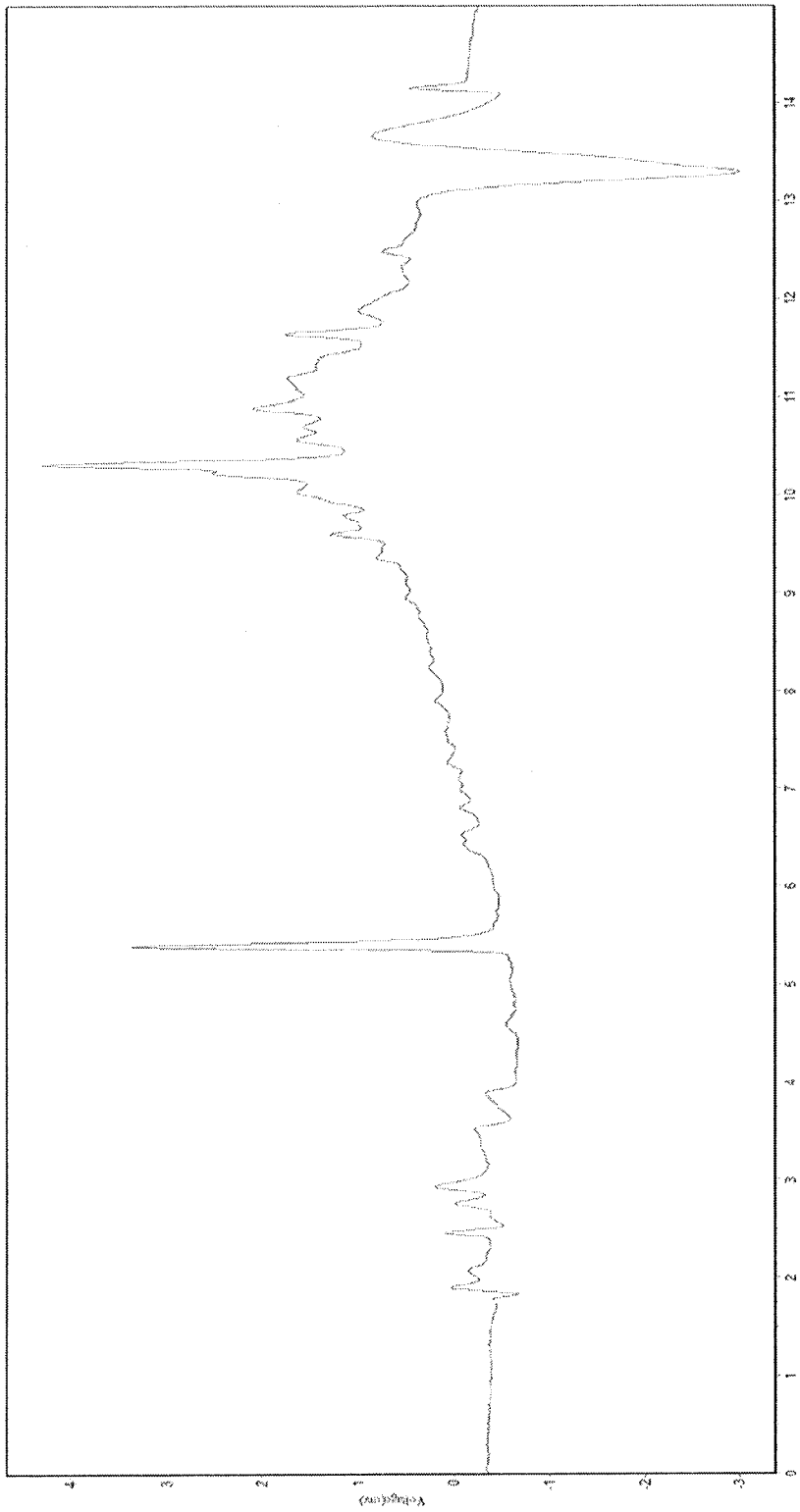

FIG. 15D

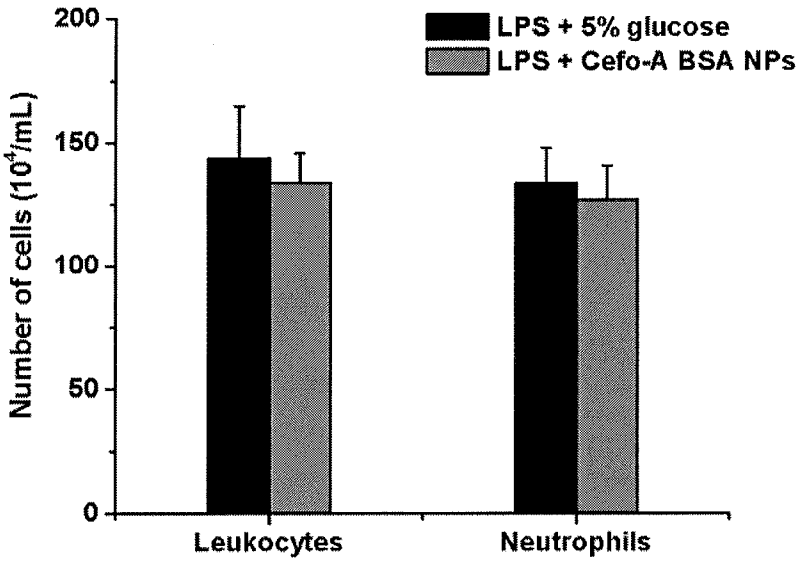

FIG. 16A

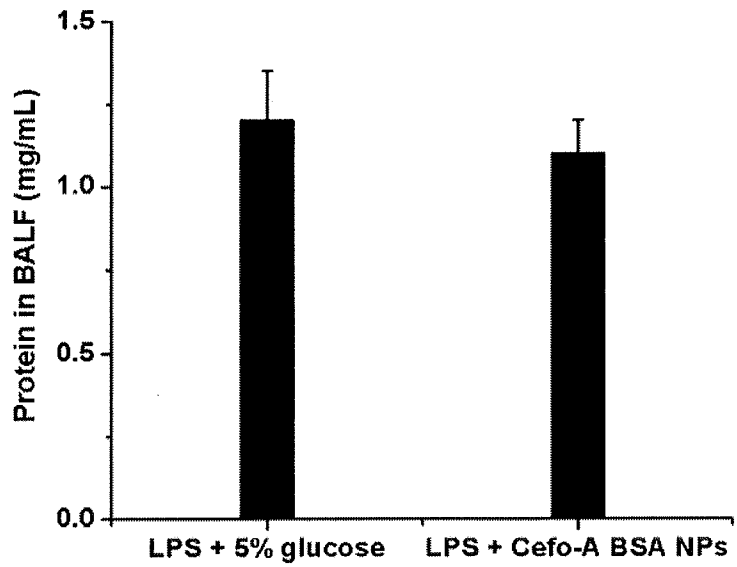

FIG. 16B

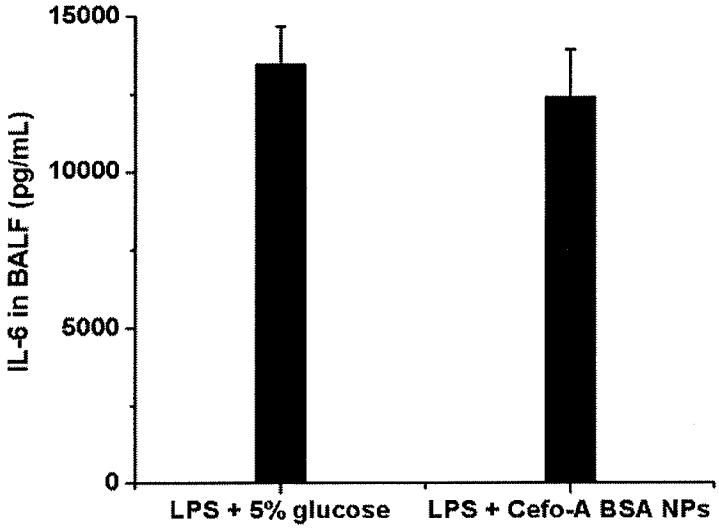

FIG. 16C

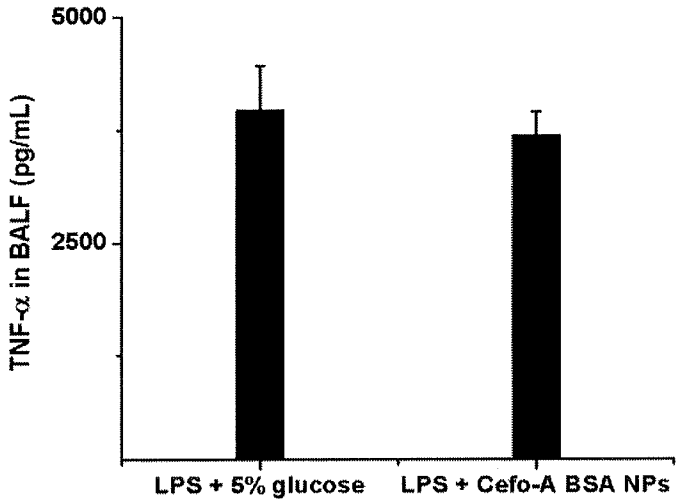

FIG. 16D

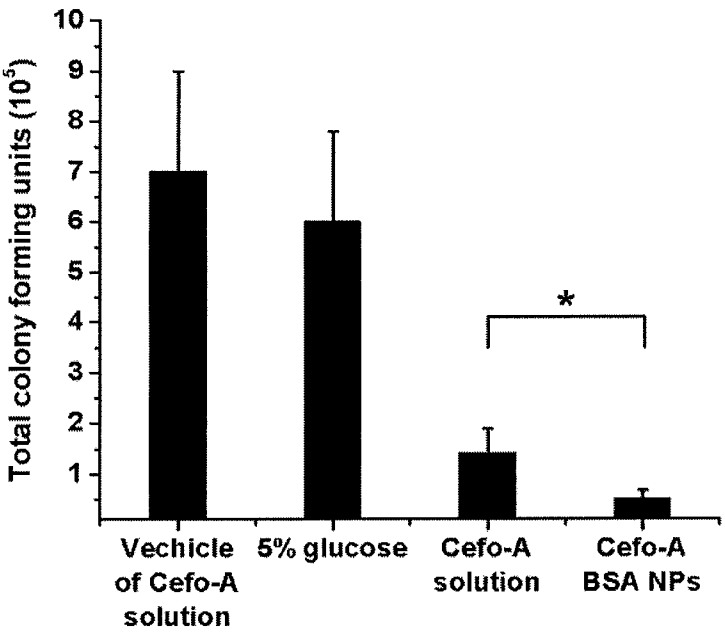
Total colony forming units (10^5)
10
9
8
7
6
5
4
3
2
1
*
Vechicle of Cefo-A solution
5% glucose
Cefo-A solution
Cefo-A BSA NPs

FIG. 16E

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/28221

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 9/14, A61K 47/42, A61K 31/337, A61K 9/107 (2016.01)
CPC - A61K 31/337, A61K 47/42, A61K 9/107, A61K 9/14, A61K 9/5169, A61K 9/19
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)
CPC: A61K31/337; A61K47/42; A61K9/107; A61K9/14; A61K9/5169; A61K9/19; Y10S977/70; B82Y5/00; A61K39/00; A61K9/5031
IPC(8): A61K 9/14, A61K 47/42, A61K 31/337, A61K 9/107 (2016.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 424/499; 514/449; 424/489; 514/15.2; 514/19.2; 424/85.4; 424/133.1; 424/450; 514/570; 424/490; 424/184.1; 424/484; 514/2.3; 424/139.1; 514/3.7; 424/85.7; 525/54.1

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Pat Base (AU BE BR CA CH CN DE DK EP ES FI FR GB IN JP KR SE TH TW US WO), Google Patent, Google Scholar; Search terms:nanoparticles albumin drug antibiotic anti-inflammatory anti-cancer diameter bovine serum denature polyethylene glycol NF-kB inhibitor TPCA-1 cefoperazone acid BSA medical medicine therapeutic biological bioactive pharmaceutical

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015/0050356 A1 (DESAI et al.) 19 February 2015 (19.02.2015) para [0002], [0010], [0016], [0041], [0103], [0224], [0255]; title	1-2, 4 and 11
X	US 2014/0294931 A1 (MOUSA et al.) 2 October 2014 (02.10.2014) para [0002], [0006]; abstract	1 and 3
X	US 2010/0151000 A1 (THOMAS et al.) 17 June 2010 (17.06.2010) para [0001], [0043], [0121], [0195]; abstract; table 2B	1 and 5-6
X	US 2015/0023912 A1 (KRATZ et al.) 22 January 2015 (22.01.2015) para [0014], [0035]; abstract;	1 and 7
X	WO 2000/066090 A1 (BIOTECH AUSTRALIA PTY LIMITED) 9 November 2000 (09.11.2000) pg 15, ln 8-31; pg 16, ln 1-10	1 and 8-9
X	US 2011/0250284 A1 (LAVIK et al.) 13 October 2011 (13.10.2011) para [0008]-[0010], [0143]	1 and 10

☐ 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
9 August 2016 (09.08.2016)	30 AUG 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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/28221

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.:
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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-11, directed to a nanoparticle composition.

Group II: Claims 12-35, directed to a method of transporting a nanoparticle composition across a blood vessel.

Group III: Claims 36-73, directed a method of treating a disease in a patient in need thereof.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

please see the continuation at the end of this form

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.:
1-11

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.

continuation of Box III (Lack of Unity)

Special Technical Feature:

Group I requires that the nanoparticle composition comprising a drug and an albumin protein, not required by Groups II and III.

Group II requires method of transporting a nanoparticle composition across a blood vessel, wherein the nanoparticle composition is transported by one or more neutrophils across a barrier of the blood vessel, not required by groups I and III.

Group III requires A method of treating a disease in a patient in need thereof, said method comprising the step of administering a nanoparticle composition to the patient, wherein the administration of the nanoparticle composition reduces one or more symptoms associated with the disease, not required by groups I and II.

Common technical features:

Groups I-III share the technical feature of a nanoparticle composition. However, these shared technical features do not represent a contribution over prior art, because the shared technical feature is being anticipated by US 2012/0328702 A1 to Edelson et al. (hereinafter Edelson). Edelson teaches a nanoparticle composition (para [0024]; administration of a nanoparticle composition comprising at least one known therapeutic agent and/or independently active biologically active agent to a patient suffering from and/or susceptible to a disease, condition, or disorder).

Groups I and II share the technical feature of a nanoparticle composition. However, these shared technical features do not represent a contribution over prior art, because the shared technical feature is being anticipated by Edelson. Edelson teaches a nanoparticle composition (para [0024]; administration of a nanoparticle composition comprising at least one known therapeutic agent and/or independently active biologically active agent to a patient suffering from and/or susceptible to a disease, condition, or disorder).

Groups I and III share the technical feature of a nanoparticle composition. However, these shared technical features do not represent a contribution over prior art, because the shared technical feature is being anticipated by Edelson. Edelson teaches a nanoparticle composition (para [0024]; administration of a nanoparticle composition comprising at least one known therapeutic agent and/or independently active biologically active agent to a patient suffering from and/or susceptible to a disease, condition, or disorder).
Groups II and III share the technical feature of administering a nanoparticle composition to the patient. However, these shared technical features do not represent a contribution over prior art, because the shared technical feature is being anticipated by Edelson. Edelson teaches of administering a nanoparticle composition to the patient (para [0024]; administration of a nanoparticle composition comprising at least one known therapeutic agent and/or independently active biologically active agent to a patient suffering from and/or susceptible to a disease, condition, or disorder).

As the shared technical features were known in the art at the time of the invention, they cannot be considered common technical features that would otherwise unify the groups. Therefore, Groups I-III lack unity under PCT Rule 13.