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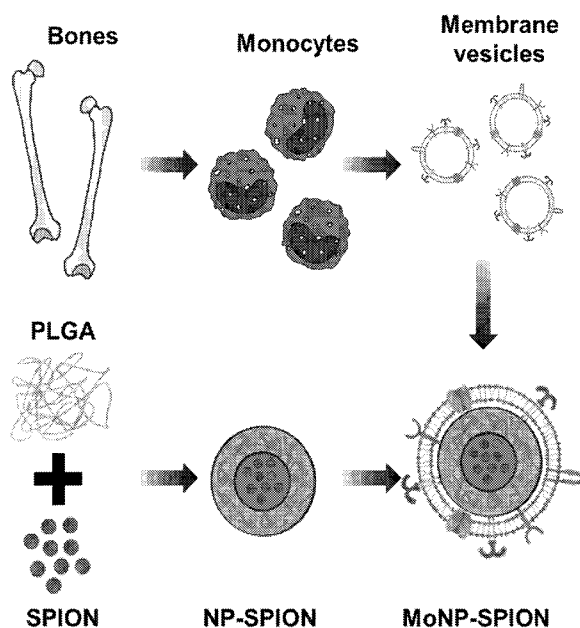


FIG. 1A

(57) Abstract: Provided herein are lipid nanoparticle complex with nanoparticles cloaked in monocyte plasma membrane. The nanoparticles of the lipid nanoparticle complex can be loaded with imaging agents. Also provided are methods of using the lipid nanoparticle complex for targeting and imaging of atherosclerosis in a subject in need thereof.

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MEMBRANE COATED NANOPARTICLES FOR IMAGING**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] The present application claims the benefit of U.S. Provisional Patent Application No. 63/544,600, entitled, “MEMBRANE COATED NANOPARTICLES FOR IMAGING” filed October 17, 2023, the content of which is hereby incorporated by reference in its entirety.

GOVERNMENT SUPPORT

[0002] This invention was made with government support under R00 HL135416 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD

[0003] The present disclosure relates to targeted nanoparticle delivery using biomimetic nanocarriers and their use in imaging disease condition, for example atherosclerosis.

BACKGROUND

[0004] Atherosclerosis, characterized by plaque deposition on arterial wall, is the predominant contributor to acute cardiovascular events, including heart attack and stroke, significantly impacting public health globally. Often, due to its long asymptomatic phase, many individuals with atherosclerosis remain undiagnosed until they suddenly experience a serious or even fatal event. Although advancements in medical imaging technologies over the past few decades have markedly improved our capabilities to diagnose, monitor disease progression, and assess treatment outcomes, the early detection of “silent” atherosclerosis, which is crucial for timely intervention, continues to be a significant challenge.

[0005] Depending on specific clinical scenarios and medical needs, a variety of imaging approaches, both noninvasive and invasive, are commonly utilized to diagnose and manage atherosclerosis. For instance, computed tomography (CT), ultrasound, and magnetic resonance imaging (MRI), are employed as first-line diagnostic tools to detect the anatomical changes in blood vessels affected by atherosclerosis. For patients presenting with symptoms, catheter-based angiography and optical coherence tomography (OCT) are typically chosen to

more precisely assess the location and extent of luminal blockages and to facilitate real-time interventions. Among these diagnostic tools, MRI is a noninvasive imaging technique that leverages magnetic fields and radio waves to measure the response of protons within water molecules in the body, producing high-contrast images for various soft tissues without exposure to ionizing radiation. In vascular applications, MRI is frequently employed to visualize abnormalities in blood vessels, such as stenosis, occlusion, and aneurysms, through a technique known as magnetic resonance angiography (MRA). When imaged with gadolinium (Gd)-based contrast agents, the signal and differentiation of tissue structures are significantly enhanced, although the toxicity of Gd remain a concern. These features make MRI especially valuable for monitoring the progression of cardiovascular diseases and evaluating the efficacy of therapeutic interventions.

[0006] Iron oxide nanoparticles have a wide range of biomedical applications, including use as carriers for drug delivery, in hyperthermia cancer treatment, and for iron replacement therapy. Particularly, those below 50 nm in diameter, often referred to as superparamagnetic iron oxide nanoparticles (SPIONs), can effectively shorten transverse relaxation time, resulting in strong T2 hypointense contrast in MR imaging, and have therefore been widely explored as MRI contrast agents. In cardiovascular imaging, while Gd-based chelates are routinely used in MRA to enhance the visualization of blood flow and luminal structures, SPIONs, through their uptake by circulating phagocytes and tissue macrophages, offer the potential to reveal inflammatory activity within the atheroma, providing valuable insights into plaque stability and the progression of atherosclerosis. However, the clinical use of SPION-enhanced MRI for atherosclerosis imaging is currently limited by their low sensitivity and specificity. Additionally, the potential uptake of SPIONs by healthy tissues raises concerns about adverse effects related to iron overload. To address these challenges, conjugating contrast agents to peptides or antibodies that target plaque-associated adhesion molecules, such as VCAM1, has shown improved sensitivity compared to untargeted SPIONs in atherosclerosis MR imaging. However, issues with ligand stability and immunogenicity still need to be resolved. Whether ligand-conjugated or not, SPIONs typically require prolonged circulation times to accumulate sufficiently for effective detection sensitivity. In this regard, a targeted carrier that efficiently deliver a high amount of imaging payload specifically to plaques could offer a more effective and safer strategy for implementing SPION-based imaging in atherosclerosis.

[0007] There is, therefore, a need for a safe and an effective method for imaging plaque development in its early stages for visualization and assessment of atherosclerosis.

SUMMARY

[0008] In some aspects the current disclosure encompasses a lipid nanoparticle complex comprising a superparamagnetic nanoparticle encapsulated with a polymer shell; and a lipid membrane coating the polymer encapsulated superparamagnetic nanoparticle.

[0009] Further provided herein is a method of producing a lipid nanoparticle complex comprising: (a) forming a superparamagnetic nanoparticle; (b) encapsulating the superparamagnetic nanoparticle with a polymer shell; (c) isolating a plasma membrane from a monocyte; (d) contacting the plasma membrane of (c) with the encapsulated superparamagnetic nanoparticle (b) to form the lipid nanoparticle complex.

[0010] In some aspects, the lipid membrane comprises a monocyte plasma membrane. In some aspects, the lipid membrane does not comprise a cytosolic protein. In some aspects, the lipid membrane comprises at least one membrane protein. In some aspects, the membrane protein is selected from an adhesion protein, a glycoprotein, a type I transmembrane protein, an integrin, CD11b, TLR4, and Na^+/K^+ -ATPase. In some aspects, the superparamagnetic nanoparticle comprises nanoparticle loaded with iron-oxide, iron cobalt, any other particle exhibiting paramagnetic properties, or a combination thereof. In some aspects, the superparamagnetic nanoparticle comprises nanoparticle loaded with iron-oxide. In some aspects, the polymer shell encapsulating the superparamagnetic nanoparticle comprises poly (lactic-co-glycolic acid) (PLGA).

[0011] In some aspects, the complex has an average hydrodynamic diameter of from about 200 to about 700 nm, about 300 to about 600 nm, or from about 200 to about 300 nm. In some aspects, the complex has an average hydrodynamic diameter of about 245 nm to about 295 nm. In some aspects, the complex has a surface charge of about -20 to about -60 mV. In some aspects, the complex has a surface charge of about -23 mV.

[0012] Further provided is a composition comprising a lipid nanoparticle complex and a carrier or excipient. The disclosure further encompasses a method of imaging atherosclerosis in a subject in need thereof, the method comprising administering to the subject a lipid nanoparticle complex or a composition thereof. The lipid nanoparticle complex or the

composition is administered by injection. In some aspects, the subject is a human. The imaging may be a magnetic resonance imaging (MRI). The method may further comprise providing an appropriate treatment to the subject.

[0013] The disclosure further encompasses a contrast enhancing agent for use in MRI comprising the lipid nanoparticle complex or a composition thereof.

BRIEF DESCRIPTION OF THE FIGURES

[0014] The application file contains at least one drawing executed in color. Copies of this patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

[0015] **FIG. 1A-1G** show MoNP-SPION exhibits an enhanced T2 relaxivity. **FIG. 1A** is a schematic illustration of the synthesis process of MoNP-SPION. **FIG. 1B** is a line graph depicting the hydrodynamic diameter NP-SPION, monocyte membrane vesicles, and MoNP-SPIO from DLS data. **FIG. 1C** is a bar graph showing DLS data of NP-SPION, monocyte membrane vesicles, and MoNP-SPION depicting PDI, and zeta-potential. $n = 3$, $*p < 0.05$. **FIG. 1D** shows representative TEM images of NP-SPION, monocyte membrane vesicle, and MoNP-SPION. Scale bar = 100 nm. **FIG. 1E** is a series of photographs showing western blot analysis of monocyte proteins: 1. crude lysate; 2. cytoplasm; 3. membranes, 4. NP-SPION, 5. MoNP-SPION. **FIG. 1F** is a series of photographs showing representative T2-weighted images of SPION, NP-SPION, and MoNP-SPION. Scale bar = 2.5 mm. **FIG. 1G** is a line graph showing linear regression analysis of relaxometry data of SPION, NP-SPION, and MoNP-SPION. $n = 5$.

[0016] **FIG. 2A** is a series of photograph showing representative T1-weighted images of MoNP-SPION, NP-SPION, and SPION.

[0017] **FIG. 2B** is a line graph depicting linear regression analysis of relaxometry data. $n = 5$.

[0018] **FIG. 3A-3I** show MoNP-SPION enables preferential uptake by inflamed endothelium while avoiding phagocytes. **FIG. 3A** is a series of photographs showing representative images of Prussian blue-stained HUVEC treated with MoNP-SPION, NP-SPION, SPION, or saline. Scale bar = 200 μm . **FIG. 3B** is a bar graph showing quantification of iron levels in HUVEC. **FIG. 3C** is a series of photographs showing representative T2-weighted MR

phantom images of HUVEC treated with MoNP-SPION, NP-SPION, SPION, or saline. Scale bar = 2.5 mm. **FIG. 3D** is a bar graph showing quantification of signal intensities (SI) of T2*-weighted phantom images. **FIG. 3E** is a series of photographs showing representative images of prussian blue-stained macrophages and monocytes treated with MoNP-SPION, NP-SPION, SPION, or saline. Left scale bar = 200 μ m. Right scale bar = 100 μ m. **FIG. 3F** is a bar graph showing quantification of iron content in macrophage and monocytes. **FIG. 3G** is a series of photographs showing representative T2-weighted MR phantoms of macrophages (left) and monocytes (right) treated with MoNP-SPION, NP-SPION, SPION, or saline. Scale bar = 2.5 mm. **FIG. 3H** is a bar graph showing quantification of macrophage and monocyte SI of T2*-weighted phantom images. **FIG. 3I** is a bar graph showing quantification of iron levels in mouse plasma. $n \geq 3$, $*p < 0.05$. Fold changes calculations are based on normalization to saline control groups.

[0019] **FIG. 4A-4D** shows MoNP-SPION administration facilitates targeted imaging of early atherosclerotic lesions. **FIG. 4A** is a schematic representation of the PL model. **FIG. 4B** is a schematic of the experimental timeline. **FIG. 4C** is a series of photographs of representative brightfield and T2*-weighted images of arterial tissue segments, from carotid bifurcation to descending aorta. Scale bar = 2.5 mm. **FIG. 4D** is a bar graph showing quantification of contrast changes in LCAs after administration of MoNP-SPION, NP-SPION, SPION, or saline. Data represented as a loss of signal normalized to the RCA; $n = 4$, $*p < 0.05$.

[0020] **FIG. 5A-5E** show MoNP-SPION induces T2* contrast within the existing plaques *in vivo*. **FIG. 5A** is a schematic diagram of the experimental timeline. **FIG. 5B** is a schematic diagram of the imaging planes. **FIG. 5C** is a series of photographs of Axial T2*-weighted images of the carotid bifurcation 2-hours post-administration of MoNP-SPION, NP-SPION, SPION, or saline, and a bar graph showing the % reduction in SI post-NP or saline administration, normalized to the pre-injection signal. **FIG. 5D** is a series of photographs showing Axial T2*-weighted images of aortic root 2-hours post-administration of MoNP-SPION, NP-SPION, SPION, or saline and a bar graph showing % reduction in SI post-NP or saline administration, normalized to the pre-injection signal. **FIG. 5E** is a series of photographs showing Prussian Blue-stained sections of the carotid artery and aortic root to identify iron presence. Scale bars: top left = 200 μ m; bottom left = 40 μ m; top right = 400 μ m; bottom right =

100 μm . $n = 4$ for MoNP-SPION, NP-SPION, and SPION groups and $n = 3$ for the saline group, $*p < 0.05$.

[0021] **FIG. 6A** is a series of Axial T1-weighted images of (A) the carotid bifurcation 2-hours post-administration of MoNP-SPION, NP-SPION, SPION, or saline. $n = 4$ for MoNP-SPION, NP-SPION, and SPION groups and $n = 3$ for the saline group, $*p < 0.05$.

[0022] **FIG. 6B** is a series of Axial T1-weighted images of aortic root 2-hours post-administration of MoNP-SPION, NP-SPION, SPION, or saline. $n = 4$ for MoNP-SPION, NP-SPION, and SPION groups and $n = 3$ for the saline group, $*p < 0.05$.

[0023] **FIG. 7A-7C** show MoNP-SPION exhibits minimal toxicity. **FIG. 7A** is a bar graph showing hemolysis assay of MoNP-SPION and SPION: data are presented as % of total maximum hemolysis, with 100% hemolysis achieved by treating mouse whole blood with water. **FIG. 7B** is a series of bar graphs showing measurement of major liver and kidney metabolites, including bilirubin, AST, ALP, ALT, albumin, and creatinine, in whole blood 24-hours post-NP or saline administration. **FIG. 7C** is a series of photographs showing representative H&E-stained sections of heart, lung, liver, kidney, and spleen from mice receiving MoNP-SPION, SPION, or saline. Scale bar = 200 μm . All graphical data: $n = 3$, $*p < 0.05$.

[0024] The drawing figures do not limit the present inventive concept to the specific aspects disclosed and described herein. The drawings are not necessarily to scale, emphasis instead being placed on clearly illustrating principles of certain aspects of the present inventive concept.

DETAILED DESCRIPTION

[0025] The current disclosure is based in part, on the identification that monocyte (Mo) membrane cloaking of nanoparticles by encapsulating nanoparticles with cell membranes from monocytes (MoNP) can enhance the ability for selective targeting and provide immune evasion of nanoparticles. Based on this, a monocyte membrane-coated nanoparticle (MoNP) platform for targeted delivery to atherosclerotic plaques was developed. This biomimetic strategy was based on the characteristic recruitment of monocytes to the vessel wall at all stages of the disease. Our recent study demonstrated that MoNPs, with their inherited membrane properties, exhibit superior stealth capabilities compared to bare polymeric NPs. More importantly, receptor integrins like VLA4 on the MoNP surface facilitate direct interactions with adhesion molecules

overexpressed on inflamed endothelial cells (ECs) lining the plaque, thereby achieving precise, atherosclerosis-specific delivery. Furthermore, our results showed that the therapeutic agent delivered via MoNP significantly reduced plaque development, while the free drug counterpart had limited therapeutic effect, highlighting the critical importance of site-specific delivery and the potential of MoNP in theranostic development for atherosclerosis. The inventors have formulated targeted contrast enhancing agents by loading SPIONs into MoNPs (MoNP-SPIONs) and evaluated their effectiveness in enhancing atherosclerosis imaging. MoNP-SPIONs were found to exhibit higher T2 relaxivity than free SPION counterpart and are more efficient at entering inflamed ECs while reducing uptake by phagocytes, compared to their constituent components. *Ex vivo* and live animal MR imaging indicated that MoNP-SPIONs preferentially accumulate in regions of atherosclerosis, producing significantly stronger hypointense signals than standalone SPIONs. MoNP-SPIONs as disclosed herein can be used for imaging and assessment of atherosclerotic regions of a vasculature and allows for early detection of atherosclerosis.

[0026] In some aspects, the current disclosure encompasses compositions comprising these lipid nanoparticle complex comprising a magnetic nanoparticle encapsulated with a polymer shell and coated with a lipid membrane. In some aspects, the superparamagnetic nanoparticle is loaded with an imaging agent, or a contrast agent used for imaging. In some aspects, the current disclosure further encompasses methods of making the lipid nanoparticle complex comprising a superparamagnetic nanoparticle disclosed herein. In some aspects, the current disclosure encompasses methods for imaging and assessment of atherosclerosis in a subject in need thereof.

a. Definitions

[0027] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this disclosure belongs. The following references provide one of skill with a general definition of many of the terms used in this disclosure: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

[0028] When introducing elements of the present disclosure or the preferred aspects(s) thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements. Wherever the terms "comprising" or "including" are used, it should be understood the disclosure also expressly contemplates and encompasses additional aspects "consisting of" the disclosed elements, in which additional elements other than the listed elements are not included.

[0029] The term "atherosclerosis" refers to a condition where plaque, which is a combination of fatty deposits, calcium, blood components, cells, and cholesterol, builds up on the inner walls of arteries throughout the body. As the plaque buildup increases, the affected artery or arteries narrows resulting in decreased blood flow through the affected area. Atherosclerosis can lead to cardiovascular events. In some aspects, atherosclerosis disclosed herein can comprise atherosclerosis found in the cardiovascular and renal systems.

[0030] Atherosclerotic plaque generally grows slowly and over time may produce a severe stenosis (a narrowing of the diameter of the artery) or may progress to total arterial occlusion. With time, the plaque becomes calcified. Some plaques are stable, but others, especially those rich in lipids and inflammatory cells (e.g., macrophages) and covered by a thin fibrous cap, may undergo spontaneous fissure or rupture, exposing the plaque contents to flowing blood. These plaques are deemed to be unstable or vulnerable and are more closely associated to the onset of an acute ischemic event. The ruptured plaque stimulates thrombosis; the thrombi may embolize, rapidly occlude the lumen to precipitate a heart attack or an acute ischemic syndrome, or gradually become incorporated into the plaque, contributing to its stepwise growth.

[0031] Atherosclerosis is characteristically silent until critical stenosis, thrombosis, aneurysm, or embolus supervenes. Initially, symptoms and signs reflect an inability of blood flow to the affected tissue to increase with demand (e.g., angina or exertion, intermittent claudication). Symptoms and signs commonly develop gradually as the atheroma slowly encroaches on the vessel lumen.

[0032] Atherosclerosis is an inflammatory disease, eventually leading to an accumulation of lipids within the artery wall. Injury to the vascular endothelium, by hypercholesterolemia, hypertension, elevated levels of homocysteine, infection, and/or other

conditions, appears to be the initiating event of atherosclerosis. This causes arterial endothelial cells to express adhesion molecules that bind various classes of leukocytes, including monocytes and T lymphocytes. Once adherent to the endothelium, chemokines (such as MCP-1) expressed within the injured arterial wall stimulate the leukocytes to migrate into the intima and thereby establish an inflammatory process ultimately leading to the development of the typical atherosclerotic plaque. This includes the transdifferentiation of monocytes into macrophages. These cells express scavenger receptors that lead to the intracellular accumulation of lipids and thereby cause the macrophages to become foam cells, the beginning of the lipid accumulation component of the atherogenic plaque. Inflammation can also contribute to the precipitation of the characteristic acute thrombotic complications of atherosclerosis. A thick fibrous cap containing collagen normally separates the lipid constituents of the plaque from the circulating blood. However, if the inflammatory cells resident within the plaque are activated, they can produce proteolytic enzymes that degrade the cap's collagen, causing it to become susceptible to erosion and rupture. Exposure of the prothrombotic constituents of the plaque to the circulating blood initiates thrombotic processes, which are further facilitated by inflammatory molecules inducing macrophages to express tissue factor, a potent trigger of thrombosis.

[0033] As used herein “Pharmaceutical composition” means a mixture of substances suitable for administering to an individual that includes a pharmaceutical agent. As used herein a pharmaceutical composition comprises lipid nanoparticle complex disclosed herein compounded with suitable pharmaceutical carriers or excipients.

[0034] As used herein “Treatment” or “therapy” of a subject refers to any type of intervention or process performed on, or the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication, condition or biochemical indicia associated with a disease.

[0035] As used herein, the term “patient”, “subject”, or “test subject” refers to any organism to which provided compound or compounds described herein are administered in accordance with the present disclosure, e.g., for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (e.g., mammals such as mice, rats, rabbits, non-human primates, humans). In an aspect, a subject is a human. In some aspects, a

subject may be suffering from, and/or susceptible to a disease, disorder, and/or condition (*e.g.*, atherosclerosis).

[0036] As used herein “animals” include a pet, a farm animal, an economic animal, a sport animal and an experimental animal, such as a cat, a dog, a horse, a cow, an ox, a pig, a donkey, a sheep, a lamb, a goat, a mouse, a rabbit, a chicken, a duck, a goose, a primate (for *e.g.*, a monkey and a chimpanzee).

[0037] The term "effective amount" as used herein is defined as the amount of the molecules of the present disclosure that are necessary to result in the desired physiological change in the cell or tissue to which it is administered. The term "therapeutically effective amount" as used herein is defined as the amount of the molecules of the present disclosure that achieves a desired effect with respect to atherosclerosis. A skilled artisan readily recognizes that in many cases the molecules may not provide a cure but may provide a partial benefit, such as alleviation or improvement of at least one symptom or parameter. In some aspects, a physiological change having some benefit is also considered therapeutically beneficial. Thus, in some aspects, an amount of molecules that provides a physiological change is considered an "effective amount" or a "therapeutically effective amount."

[0038] As used herein, the disclosure of numerical ranges by numerical endpoints includes all numbers encompassed by that range (*e.g.*, “1 to 5” includes but is not limited to 1, 1.25, 1.5, 1.75, 2, 2.3, 2.5, 2.8, 3, 3.1, 3.3, 3.8, 3.9, 4, 4.25, 4.5, 4.75 and 5). Unless otherwise indicated, all numbers used herein to express quantities, amounts, dimensions, measurements, and the like should be understood as encompassing the specific quantities, amounts, dimensions, measurements and so on, including those instances modified by the term “about.” For example, an amount disclosed herein as “about 1 mg/mL” would expressly include the amount of 1 mg/mL, and so on. Accordingly, unless indicated to the contrary, the numerical descriptions set forth herein may vary while remaining well within the teachings of the present disclosure. At the very least, each numerical value should be construed in view of the number of significant digits and by applying routine rounding techniques. As various changes could be made in the above-described cells and methods without departing from the scope of the disclosure, it is intended that all matter contained in the above description and in the examples given below, shall be interpreted as illustrative and not in a limiting sense.

[0039] As used herein, the term “nanoparticles” is defined as colloidal particles of sub-micron size of 10-1000 nanometers, such as 30-500 nanometers, or 50-350 nanometers, which may comprise a drug of interest entrapped into the matrix. Nanoparticles may be referred to as nanospheres, nanogels, nanocapsules, and micelles. The sub-micron size of nanoparticles has the advantages that they allow for cellular and tissue uptake, and they can pass through fine capillaries. Use of biodegradable materials in nanoparticle formulation may allow for sustained drug release at the target site over a period of weeks after injection. In some aspects, the nanoparticle disclosed herein can comprise an inner core which can be covered by an outer surface comprising the membrane as disclosed herein. The disclosure contemplates any nanoparticles now known and later developed that can be coated with the membrane described herein.

[0040] As used herein “plasma membrane” or “cellular membrane” refers to a biological membrane enclosing or separating structure acting as a selective barrier, within or around a cell, or a vacuole. The cellular membrane is selectively permeable to ions and organic molecules and controls the movement of substances in and out of cells. The cellular membrane comprises a phospholipid uni- or bilayer, and optionally associated proteins and carbohydrates. In some aspects, the plasma membrane refers to a membrane obtained from a naturally occurring biological membrane of a cell or cellular organelles, or one derived therefrom. As used herein, the term “naturally occurring” refers to one existing in nature. As used herein, the term “derived therefrom” refers to any subsequent modification of the natural membrane, such as isolating the cellular membrane, creating portions or fragments of the membrane, removing and/or adding certain components, such as lipid, protein or carbohydrates, from or into the membrane taken from a cell or a cellular organelle. A membrane can be derived from a naturally occurring membrane by any suitable methods. For example, a membrane can be prepared or isolated from a cell and the prepared or isolated membrane can be combined with other substances or materials to form a derived membrane. In another example, a cell or virus can be recombinantly engineered to produce “non-natural” substances that are incorporated into its membrane in vivo, and the cellular or viral membrane can be prepared or isolated from the cell or the virus to form a derived membrane.

[0041] As used herein “lipid nanoparticle” refers to a particle that comprises a plurality of (i.e. more than one) lipid molecules physically associated with each other by

intermolecular forces. In one example, the lipid nanoparticle as disclosed herein can comprise a lipid membrane coating a nanoparticle. The lipid nanoparticles may be, e.g., microspheres including but not limited to unilamellar and multilamellar vesicles, e.g., “liposomes”, lamellar phase lipid bilayers that, in some aspects, are substantially spherical and, in more particular aspects, can comprise a core, e.g., comprising iron oxide particles).

[0042] As used herein “encapsulation” or “coating” or “cloaking” is interchangeably used, and refers to transferring biological membrane, for e.g., plasma membrane onto the surface of a nanoparticle.

[0043] As used herein “magnetic nanoparticle” can include paramagnetic nanoparticles, diamagnetic nanoparticles, and ferromagnetic nanoparticles.

[0044] As used herein, “paramagnetic” refers to materials having a small and positive susceptibility to magnetic fields, which are slightly attracted by a magnetic field. In some aspects, paramagnetic materials do not retain magnetic properties when the external field is removed. These paramagnetic properties are due to the presence of some unpaired electrons and the realignment of the electron orbits caused by the external magnetic field. Examples of paramagnetic materials include, but are not limited to, magnesium, molybdenum, and lithium. The term “superparamagnetic” as used herein refers to the property of materials, which have no permanent (equiaxed) alignment of the elementary magnetic dipoles in the absence of the action of external magnetic fields. In the presence of an external magnetic field, however, superparamagnetic materials can have magnetic susceptibilities at a level similar to ferromagnetic materials. Superparamagnetism can occur when the diameter of the crystalline regions in a normally ferromagnetic substance falls below a particular critical value. In one aspect, the magnetic element may be made of a single magnetic material. Alternatively, the magnetic element may be made of a magnetic alloy. In some embodiments, the magnetic element is selected from: paramagnetic element and superparamagnetic element. In some embodiments, the magnetic element is selected from the group consisting of gadolinium, chromium, nickel, copper, iron, or manganese, iron oxides (gamma Fe_2O_3 , and Fe_3O_4), and iron hydroxides $\text{Fe}(\text{OH})_2$. In a specific aspect, the magnetic element is a superparamagnetic iron oxide nanoparticle (SPION).

[0045] As used herein “superparamagnetic iron oxide nanoparticle” as used herein are nanoparticles, which are magnetically attractable. The skilled person is aware that the

magnetic behavior of materials can be classified into five major groups: ferro-, ferri-, para-, antiferro-, and diamagnetism. Ferro- and ferrimagnetism are the basic mechanisms in permanent magnets. Permanent magnets can be magnetized by external magnetic fields and remain magnetized after the removal of the fields. Materials such as Fe (iron), Co, Ni, and their alloys, and some compounds of rare earth elements are ferromagnetic. Superparamagnetism is a form of magnetism, which appears in small ferromagnetic or ferrimagnetic particles. "Particles" as used herein are defined as particles of matter that are from 1 nm (nanometer) to 2 micrometers (micro m) in diameter. Furthermore, the skilled person is aware of iron (Fe) based particles, such as iron oxide based particles, which have been used in biological applications. For example, the particles may be iron oxide, iron (Fe), iron-cobalt, alnico or permalloy particles. In some aspects, the superparamagnetic iron-based particles may be selected from iron oxide, iron (Fe), iron cobalt, alnico, and permalloy particles. A "nanoparticle", as used herein, is a particle of matter that is between 1 nm and 1 micro m in diameter. Furthermore, the superparamagnetic iron-based particles may be superparamagnetic iron oxide nanoparticles (SPIONs), more preferably wherein the iron oxide is selected from Fe_3O_4 , $\gamma\text{-Fe}_2\text{O}_3$, CoFeO_4 or a mixture thereof, more preferably wherein the iron oxide is Fe_3O_4 . In a specific aspect, the concentration of the superparamagnetic iron-based particles is determined by atomic emission spectroscopy (AES). The iron content in a solution may, for example, be determined with Atomic Emission Spectroscopy (AES). The skilled person is also aware of other methods in order to determine the iron content in a sample. For example, the concentration of iron in a sample can also be determined photometrically.

[0046] As used herein "Magnetic Resonance Imaging" or "MRI", also known as nuclear magnetic resonance imaging (NMRI), or magnetic resonance tomography (MRT) is a medical imaging technique used to visualize internal structures of the body. Body tissue contains large amounts of water, and hence protons (^1H nuclei) since each water molecule has two hydrogen nuclei (protons). The protons can be aligned in a strong magnetic field generated by a powerful magnet of an MRI scanner. When a person is inside this magnetic field, the average magnetic moment of many protons becomes aligned with the direction of the field. A radio frequency current is briefly turned on, producing a varying electromagnetic field. This electromagnetic field has just the right frequency, known as the resonance frequency, to be absorbed and flip the spin of the protons in the magnetic field. After the electromagnetic field is

turned off, the spins of the protons return to thermodynamic equilibrium and the bulk magnetization becomes re-aligned with the static magnetic field. During this relaxation, a radio frequency signal (electromagnetic radiation in the RF range) is generated, which can be recorded and measured with receiver coils of the MRI scanner. The recorded information is used to construct an image of the scanned area of the body. The energy absorbed by the body during MRI is small, and it is thought that MRI can be used safely and repeatedly for diagnosis. MRI provides good contrast between the different soft tissues of the body and can be used to image every part of the body, particularly for tissues with many hydrogen nuclei and little density contrast, such as the brain, muscle, connective tissue and most tumors, because the composition of these tissues influences the relaxation of the protons which are imaged. However, macrophages look quite similar to surrounding tissues when imaged with MRI.

[0047] As used herein “T2*-weighted imaging” is an MRI sequence to quantify observable or effective T2 (referred to as T2* or “T2-star”). T2* can be considered an “observed” or “effective” T2, whereas T2 may be considered the “natural” or “true” T2 of the tissue being imaged. T2* is always less than or equal to T2. It is known that T2* results principally from inhomogeneities in the main magnetic field. These inhomogeneities may be the result of intrinsic defects in the magnet itself or from susceptibility-induced field distortions produced by the tissue or other materials placed within the field. Certain MR imaging sequences using gradient echoes, which are called T2*-weighted.

b. Mo membrane-coated iron oxide-loaded nanoparticles

[0048] In some aspects, the current disclosure encompasses a lipid nanoparticle complex comprising a nanoparticle encapsulated with a polymer shell; and a lipid membrane coating the polymer encapsulated nanoparticle. In such aspects, the nanoparticle comprises an imaging agent or a contrast agent. In some aspects, the contrast agent is a metallic contrast agent.

[0049] In some aspects, the nanoparticles disclosed herein comprise a core-shell structure. In some aspects, the nanoparticles can be made from a wide range of materials. The nanoparticle is preferably composed of a material suitable for biological use.

[0050] In one aspect, the core of the nanoparticle may comprise an imaging agent or a contrast enhancing agent. Any imaging or contrast agent can be contemplated for use with the lipid nanoparticle complex disclosed herein, such as for example, agents used in magnetic

resonance imaging (MRI), positron emission tomography (PET), computer tomography (CT), magnetic spectroscopy, X-ray, and ultrasonic imaging.

[0051] In some aspects, the core of the nanoparticle comprise a contrast enhancing agent. The contrast enhancing agent can be a negative contrast agent. The contrast agent can be a metallic contrast agent. The metallic contrast agent can be selected from the group consisting of Gd(III), Mn(II), Mn(III), Cr(II), Cr(III), Cu(II), Fe (III), Pr(III), Nd(III) Sm(III), Tb(III), Yb(III) Dy(III), Ho(III), Eu(II), Eu(III), and Er(III), Indium (In), Technetium (Tc), and Barium. In addition, the metallic contrast agents could include crystals and other particulate materials (oxides, quantum dots, etc.).

[0052] In some aspects, the contrast enhancing agent can be a non-metallic contrast agent. The non-metallic contrast agent may preferably be selected from the group consisting of Iodine (I), Bromine, Fluorescein, Carboxyfluorescein, Calcein, F^{18} , Xe^{133} , I^{125} , I^{131} , I^{123} , P^{32} , Tl^{201} , K^{42} , In^{111} , Fe^{59} , Tc^{99} , Cr^{51} , Ga^{67} , CU^{64} , Rb^{82} , C^{11} , N^{13} , O^{15} , Mo^{99} , Kr^{81} , and Dy^{165} .

[0053] In some aspects, the nanoparticles comprise an iron oxide core. The iron oxide may be a superparamagnetic iron oxide and the nanoparticles are superparamagnetic iron oxide nanoparticles (SPIONs). The core is preferably an iron oxide particle such as a crystal, for example a nanocrystal of iron oxide. A crystal may be a crystal that has a reverse spinel structure. Suitable iron oxides are oxides of Fe(II) such as FeO and FeO₂, oxides of Fe(III) such as α -Fe₂O₃ (hematite), β -Fe₂O₃, γ -Fe₂O₃ (maghemite), and ϵ -Fe₂O₃, and mixed oxides of Fe(II) and Fe(III) such as Fe₃O₄ (magnetite), Fe₄O₅, Fe₅O₆, Fe₅O₇, Fe₂₅O₃₂, and Fe₁₃O₁₉. Preferred iron oxides are oxides of Fe(III) and mixed oxides of Fe(II) and Fe(III). Other iron oxides include Fe₂O₃, Fe₃O₄, Fe₄O₅, Fe₅O₆, and Fe₅O₇. Iron oxides can also be maghemite and magnetite. A magnetite may be nonstoichiometric magnetite.

[0054] In one aspect, the diameter of the core, for example SPION, can be from about 10 nm to about 200 nm. For example, the size of the SPION core can be from about 12 nm to about 180 nm; from about 15 nm to about 150 nm; from about 20 nm to about 125 nm; from about 25 nm to about 100 nm; from about 30 nm to about 75 nm; from about 35 nm to about 70 nm; from about 40 nm to about 65 nm; from about 45 nm to about 60nm; from about 50 to about 55 nm. In certain aspects, the diameter of the core may be about 10 nm to about 20 nm.

[0055] In some aspects, the nanoparticles comprises a coating or an encapsulation. In one aspect, the nanoparticle is coated or encapsulated with a polymer. In some

aspects, the nanoparticle disclosed herein is encapsulated with a shell comprising a biocompatible and/or a synthetic material including but not limited to, poly(lactic-co-glycolic acid) (PLGA), polylactic acid, polyglycolic acid, polycaprolactone, polylysine, polyglutamic acid, and any other suitable synthetic material or the like. In some aspects, the shell of the nanoparticle disclosed herein comprises poly(D, L-lactide-co-glycolide), or PLGA.

[0056] The nanoparticles disclosed herein can be prepared using any suitable methods available in the art. Non-limiting examples of methods that may be used for preparing nanoparticle disclosed herein include emulsion-solvent evaporation, emulsification-evaporation, nanoprecipitation or solvent displacement, solvent diffusion, and phase-inversion.

[0057] The nanoparticle disclosed herein can have any suitable shape. For example, the present nanoparticle and/or its inner core can have a shape of sphere, circular, square, rectangle, triangle, circular disc, cube-like shape, cube, rectangular parallelepiped (cuboid), cone, cylinder, prism, pyramid, right-angled circular cylinder and other regular or irregular shape.

[0058] The nanoparticle disclosed herein can have any suitable size. In some aspects, the nanoparticle has a hydrodynamic size of about 50-500 nm. For example, the nanoparticles may have a hydrodynamic size of about 50-100 nm, or about 100-150 nm, or about 150-200 nm, or about 200-250 nm, or about 250-300 nm, or about 300-350 nm, or about 350-400 nm, or about 400-450 nm or about 450-500 nm. Particle size can be affected by the polymer concentration; higher particles are formed from higher polymer concentrations. In certain aspects, the nanoparticles disclosed herein can have a hydrodynamic size of about 200 nm, about 210 nm, about 220 nm, about 230 nm, about 240 nm, about 250 nm, about 260 nm, about 270 nm, about 280 nm, about 290 nm, about 300 nm, about 310 nm, or about 320 nm. In some aspects, the nanoparticles disclosed herein can have a hydrodynamic size of about 250 nm. In some aspects, the nanoparticles disclosed herein can have a hydrodynamic size of about 270 nm. In some aspects, the nanoparticles disclosed herein can have a hydrodynamic size of about 300 nm.

[0059] The encapsulated nanoparticles, i.e. SPIONs are further cloaked, coated, or encapsulated in lipid membrane to form a lipid nanoparticle complex. In some aspects, the lipid membrane can be any suitable biological membrane, for example a plasma membrane. In certain aspects, the nanoparticle cloaked, coated, or encapsulated with a naturally occurring

cellular membrane and/or further comprises a synthetic membrane. In certain aspects, the plasma membrane is derived from a blood cell (e.g., red blood cell (RBC), white blood cell (WBC), or platelet). In other aspects, the plasma membrane is derived from an immune cell (e.g., macrophage, monocyte, B-cell, or T-cell). In particular aspects, the plasma membrane is from a blood cell. In some aspects, the plasma membrane is from a leucocyte or white blood cell. In some aspects, the plasma membrane is derived from a monocyte. In some aspects, the plasma membrane is derived from a mammalian monocyte. In some aspects, the plasma membrane is derived from a mouse monocyte or a human monocyte. The plasma membrane derived may be a membrane vesicle.

[0060] The SPIONs encapsulated with a polymer, for example, PLGA, is coated with a plasma membrane derived from a monocyte.

[0061] In some aspects, the monocytes disclosed herein can be isolated from sources, non-limiting examples of which include bone marrow, blood sample, and peripheral blood mononuclear cell (PBMC). In some aspects, the monocytes disclosed herein can be isolated from bone marrow. In some aspects, the plasma membrane can be derived from the monocytes isolated from the subject that is need of a treatment, as disclosed herein. In certain aspects, the plasma membrane is isolated from a cell of the same species of the subject. In some aspects, monocytes isolated from a donor can be used. In such aspects, the monocytes may be collected from a single donor or multiple donors. In some aspects, the donor may be a matched donor. In such aspects, the donor may have the same blood type as that of the subject. In some aspects, the plasma membrane may be derived from a monocyte cell line, for e.g., THP-1, HL-60, SC cell line, or U937.

[0062] In some aspects, monocytes disclosed herein is a classical monocyte. In such aspects, monocytes comprise cells that express the markers: Ly6c, CD11b, and CCR2 (for e.g., Ly6c⁺, CD11b⁺, and CCR2⁺) and that do not express the marker: CD45R (for e.g., CD45R⁻)

[0063] In some aspects, the lipid membrane disclosed herein maintains natural structural integrity and activity of the plasma membrane. In some aspects, the structural integrity of the plasma membrane includes primary, secondary, tertiary or quaternary structure of the plasma membrane, and/or the activity of the cellular membrane which includes, but is not limited to, binding activity, receptor activity, signaling pathway activity, and any other activities a normal naturally occurring plasma membrane. In some aspects, the lipid bilayer structure and at

least some of the associated membrane proteins embedded therewith in the disclosed plasma membrane are intact. In some aspects, the lipid nanoparticle complex or the cloaked nanoparticle mimics a cell surface. In some aspects, the lipid membrane mimics the plasma membrane of a monocyte.

[0064] In some aspects, the plasma membrane disclosed herein comprises at least one membrane protein. In some aspects, the membrane protein is an adhesion protein, a glycoprotein, a type I transmembrane protein, an integrin (for example VLA4), CD11b, TLR4, Na⁺/K⁺-ATPase, or any combination thereof. In some aspects, the plasma membrane disclosed herein comprises an adhesion protein. In some aspects, the plasma membrane disclosed herein comprises a glycoprotein. In some aspects, the plasma membrane disclosed herein comprises a type I transmembrane protein. In some aspects, the plasma membrane disclosed herein comprises an adhesion protein, a glycoprotein, or a type I transmembrane protein, or any combination thereof.

[0065] Membranes may also comprise other agents that may or may not increase an activity of the lipid nanoparticle complex. In some aspects, functional groups such as antibodies and aptamers can be added to the outer surface of the membrane to enhance the activity or site targeting of the lipid nanoparticle complex.

[0066] In certain aspects, the lipid nanoparticle complex of the present disclosure is biocompatible and/or biodegradable. In some aspects, the nanoparticle of the lipid nanoparticle complex may comprise biodegradable and biocompatible polymer, for e.g. poly DL-lactide-co-glycolide (PLGA), polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL), polylysine, and/or polyglutamic acid. In some aspects, the lipid nanoparticle complex of the present disclosure comprises the plasma membrane derived from a monocyte and an inner core encapsulated with PLGA.

[0067] In some aspects, the lipid nanoparticle complex or the cloaked nanoparticle can substantially lack constituents of the cell from which the lipid membrane is derived or its constituents. In certain aspects, the cloaked nanoparticle substantially lacks cytoplasm, nucleus and/or cellular organelles of the cell from which the plasma membrane is derived. In some aspects, the lipid membrane does not comprise a cytosolic protein of the cell from which the plasma membrane is derived. For example, the present lipid nanoparticle complex can lack, in terms of types and/or quantities, at least 10%, 20%, 30%, 40%, 50%, 60%,

70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% the constituents of the cell from which the plasma membrane is derived.

[0068] In some aspects, the lipid nanoparticle complex of the present disclosure comprises the plasma membrane derived from a monocyte coating a PLGA encapsulated nanoparticle loaded with contrast enhancing agent, the lipid nanoparticle complex substantially lacking constituents of the cell from which the lipid membrane is derived. In some aspects, the lipid nanoparticle complex of the present disclosure comprises the plasma membrane derived from a monocyte coating a PLGA encapsulated nanoparticle loaded with contrast enhancing agent, the lipid nanoparticle complex substantially lacking cytosolic proteins from which the lipid membrane is derived.

[0069] In some aspects, the lipid nanoparticle complex disclosed herein have a surface charge of about -15 to -60 mV. In some aspects, the lipid nanoparticle complex has a surface charge of about -15, or -16, or -17, or -18, or -19, or -20, or -30, or -40, or -50, or -60 mV. In some exemplary aspects, the complex has a surface charge of about -23 mV.

[0070] The lipid nanoparticle complex disclosed herein can have any suitable size. In some aspects, the lipid nanoparticle complex can have a hydrodynamic size of about 50-500 nm. In some aspects, the lipid nanoparticle complex has a hydrodynamic size of about 50-100 nm, or about 100-150 nm, or about 150-200 nm, or about 200-250 nm, or about 250-300 nm, or about 300-350 nm, or about 350-400 nm, or about 400-450 nm or about 450-500 nm. Particle size can be affected by the polymer concentration; higher particles are formed from higher polymer concentrations. In some aspects, the lipid nanoparticle complex disclosed herein can have a hydrodynamic size of about 200 nm. In some aspects, the lipid nanoparticle complex disclosed herein can have a hydrodynamic size of about 250 nm. In some aspects, the lipid nanoparticle complex disclosed herein can have a hydrodynamic size of about 280 nm. In some aspects, the lipid nanoparticle complex disclosed herein can have a hydrodynamic size of about 245 nm to about 295 nm.

[0071] In some aspects, the lipid nanoparticle complex disclosed herein has increased stability. In some aspects, the present lipid nanoparticle complex is stable at -20°C for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days. In some aspects, the lipid nanoparticle disclosed herein is stable at least for 4 days at -20°C. Stability of nanoparticles can

be assessed for e.g., by assessing the particle aggregation, change in particle size and/or dispersity of the nanoparticles, using known methods in art.

[0072] In some aspects, the lipid nanoparticle complex disclosed herein has decreased toxicity. Toxicity of nanoparticles can be assessed for e.g., by assessing the hemolysis or organ toxicity via histological examination, or by quantifying weight loss in a subject. In some aspects, the present lipid nanoparticle complex can have at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% less toxicity, for e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% less hemolysis, or less than 80%, 70%, 60%, 50%, 40%, 30%, 20%, or 10% weight loss in a subject, compared to the weight at the time of or before administration of the lipid nanoparticle complex.

[0073] In some aspects, the lipid nanoparticle complex disclosed herein has enhanced uptake in endothelial cells compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. In some aspects, the present lipid nanoparticle complex can have at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% enhanced uptake in endothelial cells compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. In some aspects, lipid nanoparticle complex can have at least 10% enhanced uptake in endothelial cells compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. In some aspects, lipid nanoparticle complex can have at least 40% enhanced uptake in endothelial cells compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. Uptake of nanoparticles can be assessed using any known method in the art including cell staining, fluorescence microscopy and flow cytometry.

[0074] In some aspects, the lipid nanoparticle complex disclosed herein has enhanced uptake in inflamed endothelial cells compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. In some aspects, the present lipid nanoparticle complex can have at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% enhanced uptake in inflamed endothelial cells compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. In some aspects, lipid nanoparticle complex can have at least 10% enhanced uptake in inflamed endothelial cells compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. In some aspects, lipid nanoparticle complex can have at least 40% enhanced uptake in inflamed endothelial cells compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane.

Inflamed endothelial cells can be identified using known biomarkers including vascular cell adhesion protein-1 (VCAM-1) and uptake of nanoparticles can be assessed using any known method in the art including cell staining, fluorescence microscopy and flow cytometry.

[0075] In further aspects, the lipid nanoparticle complex disclosed herein has enhanced uptake in endothelial cells lining atherosclerotic vasculature compared to quiescent endothelial cells. In some aspects, the present lipid nanoparticle complex can have at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% enhanced uptake in endothelial cells lining atherosclerotic vasculature compared to quiescent endothelial cells. In some aspects, lipid nanoparticle complex can have at least 10% enhanced uptake in endothelial cells lining atherosclerotic vasculature compared to quiescent endothelial cells. In some aspects, lipid nanoparticle complex can have at least 40% enhanced uptake in endothelial cells lining atherosclerotic vasculature compared to quiescent endothelial cells. Inflamed endothelial cells can be identified using known biomarkers including vascular cell adhesion protein-1 (VCAM-1) and uptake of nanoparticles can be assessed using any known method in the art including cell staining, fluorescence microscopy and flow cytometry.

[0076] In one aspect, the lipid nanoparticle complex disclosed herein has reduced uptake by phagocytes. In some aspects, the present lipid nanoparticle complex can have at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% reduced uptake by phagocytes.

c. Compositions

[0077] Further provided herein is a composition comprising the disclosed lipid nanoparticle complex. In some aspects, the composition comprises a lipid nanoparticle complex comprising a PLGA encapsulated nanoparticle loaded with contrast enhancing agent coated with a plasma membrane derived from a monocyte. In some aspects, the composition comprises a lipid nanoparticle complex comprising a PLGA encapsulated nanoparticle loaded with iron oxide coated with a plasma membrane derived from a monocyte. In some aspects, provided herein is a composition comprising an effective amount of disclosed lipid nanoparticle complex. In some aspects, the composition used for diagnosis, assessment, and imaging of atherosclerosis.

[0078] In certain aspects, compositions disclosed herein may further comprise one or more diluent(s), excipient(s), and/or carrier(s). As used herein, an acceptable diluent,

excipient, or carrier, refers to a material suitable for administration to a subject without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained. Diluents, carriers, and excipients can include, but are not limited to, physiological saline, Ringer's solution, phosphate solution or buffer, buffered saline, and other carriers known in the art.

[0079] In some aspects, compositions herein may also include stabilizers, antioxidants, colorants, other medicinal or pharmaceutical agents, carriers, adjuvants, preserving agents, stabilizing agents, wetting agents, emulsifying agents, solution promoters, salts, solubilizers, antifoaming agents, antioxidants, dispersing agents, surfactants, or any combination thereof. Herein, the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols. Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition, which is incorporated herein by reference.

[0080] In certain aspects, the present disclosure provides compositions formulated for one or more routes of administration. Suitable routes of administration may, for example, include intravenous, intracranial, intrathecal, subcutaneous, intranasal route, cranial, transmucosal, trans-nasal, transcranial, intracerebroventricular, intestinal, and/or parenteral delivery. In some aspects, compositions herein formulated can be formulated for parenteral delivery. In some aspects, compositions herein formulated can be formulated intramuscular, subcutaneous, intramedullary, intravenous, intraperitoneal, intracranial and/or intranasal injections.

[0081] In certain aspects, one may administer a composition disclosed herein in a local or systemic manner, for example, via local injection of the composition directly into a tissue region of a patient. In some aspects, the composition disclosed herein can be administered parenterally, e.g., by intravenous injection, intracerebroventricular injection, intra-cisterna magna injection, intra-parenchymal injection, or a combination thereof. In some aspects, the composition disclosed herein can administered to subject as disclosed herein. In some aspects, a composition disclosed herein can administered to human subject. In some aspects, a composition

disclosed herein can administered to a human subject via two or more administration routes. In some aspects, the combination of administration routes by be intracerebroventricular injection and intravenous injection; intrathecal injection and intravenous injection; intra-cisterna magna injection and intravenous injection; and/or intra-parenchymal injection and intravenous injection.

[0082] In certain aspects, compositions of the present disclosure may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

[0083] In certain aspects, compositions for use in accordance with the present disclosure thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. For injection, the active ingredients of a pharmaceutical composition herein may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, physiological salt buffer, or any combination thereof.

[0084] In one aspect, the lipid nanoparticle complex is formulated into a composition for use in procedures including medical imaging or diagnostic imaging. In such aspects, the lipid nanoparticle complex may be used as a contrast enhancing agent. Imaging techniques can include, but not limited to ultrasound, radiography, magnetic resonance, nuclear medicine, photo acoustic, thermography, and tomography (for example, positron emission tomography, computed tomography). The composition may be formulated with a suitable carrier that is appropriate for the specific imaging technique used. For example, the lipid nanoparticle complex can be formulated into a solution for administration into a subject, by preparing the lipid nanoparticle complex, in physiologically compatible solutions non-limiting examples of which include saline, water such as Milli-Q-water, and Aqua regia. Such formulations can be formulated for administration, for example through injection.

[0085] In a specific aspect, the lipid nanoparticle complex, or composition comprising the lipid nanoparticle complex may be used as a contrast enhancing agent for procedures by magnetic resonance, such as for example, magnetic resonance imaging. In specific aspects, the lipid nanoparticle complex or a composition thereof is for use in detection and/or

monitoring of magnetic resonance imaging. The lipid nanoparticle complex, or composition comprising the lipid nanoparticle complex may further used as a contrast enhancing agent for target specific imaging, such as for example imaging atherosclerosis.

d. Method of Making the Lipid Nanoparticle Complex

[0086] In some aspects, the current disclosure also encompasses methods of preparing the lipid nanoparticle complex described herein.

[0087] The nanoparticle lipid complexes may be prepared using a process as depicted in **FIG. 1A**. In general, the method comprises (a) forming a superparamagnetic nanoparticle; (b) encapsulating the superparamagnetic nanoparticle with a polymer shell; (c) isolating a plasma membrane from a monocyte; and (c) contacting the plasma membrane of (c) with the encapsulated superparamagnetic nanoparticle (b) to form the lipid nanoparticle complex.

[0088] As indicated, methods of preparing the complexes described herein first comprise preparing a nanoparticle. As used herein, a nanoparticle refers to a roughly spherical shaped unit that self-assembles under the appropriate conditions from an amphiphilic material so that the core is hydrophobic, and the corona is hydrophilic. Nanoparticles may be prepared according to standard methods in the art.

[0089] In some aspects, the disclosed nanoparticle can comprise and/or can be loaded with an imaging agent or a contrast enhancing agent. In some aspects, the contrast enhancing agent is a metallic contrast agent.

[0090] In some aspects, the nanoparticle comprises an iron oxide. In some aspects, the nanoparticle is a superparamagnetic nanoparticle. In some aspects, the superparamagnetic nanoparticle comprises nanoparticle loaded with iron-oxide. In further aspects, the nanoparticle is encapsulated with a polymer shell. To form iron oxide nanoparticles, i.e., SPIONs, iron oxide (II, III) nanoparticles, are suspended in pure water, and a polymer solution, for example PLGA is slowly added and dispersed for example with a probe sonicator to create the initial water-in-oil (W/O) emulsion. The W/O emulsion is then added dropwise to a polyvinyl-alcohol (PVA) solution and further sonicated to form the W/O/W emulsion, followed by addition of a PVA solution. After solvent evaporation, the mixture is washed and centrifuged to collect nanoparticle comprising iron-oxide (NP-SPION).

[0091] In further aspects, a plasma membrane or a membrane vesicle is isolated from an immune cell (e.g., a monocyte). The isolation of said plasma membrane can be performed using a variety of methods including agitation, introduction of a detergent, lysing, etc.

[0092] Finally, the plasma membrane isolated is contacted with the prepared nanoparticle. This may occur in a suitable composition or medium. The plasma membrane is mixed with the nanoparticle comprising iron oxide to form the lipid nanoparticle complex. In certain aspects, the method may comprise exerting exogenous energy on the combination i.e., isolated plasma membrane and nanoparticle (for e.g., nanoparticle core). In certain aspects, the exogenous energy is a mechanical energy exerted by extrusion. In other aspects, the exogenous energy is an acoustic energy exerted by sonication. In yet other aspect, the exogenous energy is a thermal energy exerted by heating.

e. Uses

[0093] Methods for imaging and assessment of atherosclerosis by administering the lipid nanoparticle complex according to the present disclosure is further provided. In such aspects, the method comprises administering the subject in need thereof lipid nanoparticle complex with or without a carrier, adjuvant, or excipient.

[0094] In some aspects of the method, the lipid nanoparticle complex comprises a plasma membrane derived from a monocyte and an inner core comprising PLGA, the lipid nanoparticle complex substantially lacking constituents of the cell from which the lipid membrane is derived. In further aspects, the lipid nanoparticle complex comprises a plasma membrane derived from a monocyte and an inner core comprising PLGA, the lipid nanoparticle complex substantially lacking cytosolic proteins from which the lipid membrane is derived.

[0095] In some aspects, the lipid nanoparticle complex or compositions thereof provided herein can be used for non-invasive imaging and/or assessment of atherosclerosis. Non-invasive imaging techniques include magnetic resonance imaging (MRI), positron emission tomography (PET), computer tomography (CT), magnetic spectroscopy, X-ray, and ultrasonic imaging. In such aspects, the lipid nanoparticle complex can be adapted to encapsulate various imaging agents or contrast agents for targeted imaging across different modalities, including but not limited to iron oxide nanoparticles for MRI, fluorodeoxyglucose (FDG) for

PET imaging, or iodine-based agents for CT scans. In some aspects, the lipid nanoparticle complex or compositions thereof is used for MRI.

[0096] In some aspects, the lipid nanoparticle complex or compositions thereof provided herein can be used as contrast-enhancing agents for MRI. Accordingly, methods are provided for use of the lipid nanoparticle complex or compositions thereof as MRI contrast enhancing agents, for example in T2-weighted MRI imaging. In some aspects, the lipid nanoparticle complex or compositions thereof, can enhance the T1 (longitudinal) or T2 (transverse) weighted images or both. In some aspects, lipid nanoparticle complex or compositions thereof provided herein can increase the resolution of MRI imaging and/or increase the accumulation of contrast agent in cells or tissue.

[0097] In some aspects if the method, the lipid nanoparticle complex disclosed herein has enhanced uptake in endothelial cells of a subject compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. In some aspects, the administration of the present lipid nanoparticle complex can have at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% enhanced uptake in endothelial cells compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. In some aspects, the administration of the lipid nanoparticle complex can have at least 10% enhanced uptake in endothelial cells of a subject compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. In some aspects, the lipid nanoparticle complex can have at least 40% enhanced uptake in endothelial cells of a subject compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. Uptake of nanoparticles can be assessed using any known method in the art including cell staining, fluorescence microscopy and flow cytometry.

[0098] In some aspects of the method, the lipid nanoparticle complex disclosed herein has enhanced uptake in inflamed endothelial cells of a subject compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. In some aspects, the lipid nanoparticle complex can have at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% enhanced uptake in inflamed endothelial cells of a subject compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. In some aspects, the lipid nanoparticle complex can have at least 10% enhanced uptake in inflamed endothelial cells of a subject compared to nanoparticles not cloaked, encapsulated or coated with

a plasma membrane. In some aspects, the lipid nanoparticle complex can have at least 40% enhanced uptake in inflamed endothelial cells of a subject compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. Inflamed endothelial cells can be identified using known biomarkers including vascular cell adhesion protein-1 (VCAM-1) and uptake of nanoparticles can be assessed using any known method in the art including cell staining, fluorescence microscopy and flow cytometry.

[0099] In further aspects of the method, the lipid nanoparticle complex disclosed herein has enhanced uptake in endothelial cells lining atherosclerotic vasculature of a subject compared to quiescent endothelial cells. In some aspects, the present lipid nanoparticle complex can have at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% enhanced uptake in endothelial cells lining atherosclerotic vasculature of a subject compared to quiescent endothelial cells. In some aspects, the lipid nanoparticle complex can have at least 10% enhanced uptake in endothelial cells lining atherosclerotic vasculature in a subject compared to quiescent endothelial cells. In some aspects, the lipid nanoparticle complex can have at least 40% enhanced uptake in endothelial cells lining atherosclerotic vasculature in a subject compared to quiescent endothelial cells. Inflamed endothelial cells can be identified using known biomarkers including vascular cell adhesion protein-1 (VCAM-1) and uptake of nanoparticles can be assessed using any known method in the art including cell staining, fluorescence microscopy and flow cytometry.

[00100] In further aspects, the disclosure encompasses personalized method of imaging whereby the lipid nanoparticle complex is tailored to individual patients with reduced risk of immunogenicity by using their own plasma membranes for coating nanoparticles of the lipid nanoparticle complex. In such aspects, cells for e.g., monocytes are isolated from the individual. Cells such as monocytes can be isolated from a bone marrow sample provided by the individual. The plasma membrane then can be isolated using methods disclosed herein or using other methods known in the art. The isolated plasma membrane can be used to make lipid nanoparticle complex of the present disclosure. The lipid nanoparticle complex can then be administered to the individual for imaging and assessment of atherosclerosis.

[00101] Any suitable mode of administration can be used for the lipid nanoparticle complex or compositions thereof provided herein for imaging. Exemplary modes include, but are not limited to, injection. Other modes include, without limitation, intradermal, subcutaneous

(s.c., s.q., sub-Q, Hypo), intramuscular (i.m.), intraperitoneal (i.p.), intraarterial, intramedullary, intracardiac, intra-articular (joint), intrasynovial (joint fluid area), intracranial, intraspinal, and intrathecal (spinal fluids). Any known device useful for parenteral injection or infusion of the formulations can be used to affect such administration.

[00102] In various aspects, a subject in need thereof can be having, suspected of having, or at risk of having atherosclerosis. In various aspects, a subject in need thereof can have one or more symptoms or risk factors for atherosclerosis.

[00103] In various aspects, a suitable subject includes a human, a livestock animal, a companion animal, a lab animal, or a zoological animal. In one aspect, the subject may be a rodent, e.g., a mouse, a rat, a guinea pig, etc. In another aspect, the subject may be a livestock animal. Non-limiting examples of suitable livestock animals may include pigs, cows, horses, goats, sheep, llamas and alpacas. In yet another aspect, the subject may be a companion animal. Non-limiting examples of companion animals may include pets such as dogs, cats, rabbits, and birds. In yet another aspect, the subject may be a zoological animal. As used herein, a “zoological animal” refers to an animal that may be found in a zoo. Such animals may include non-human primates, large cats, wolves, and bears. In a specific aspect, the animal is a laboratory animal. Nonlimiting examples of a laboratory animal may include rodents, canines, felines, and non-human primates. In certain aspects, the animal is a rodent. Non-limiting examples of rodents may include mice, rats, guinea pigs, etc. In certain aspects, the subject is a human.

[00104] The present disclosure provides a method of diagnostic imaging of a subject in need thereof. The method includes administering to the subject the lipid nanoparticle complex, or composition comprising the lipid nanoparticle complex and imaging a body part or a tissue of the subject. The method further comprises performing an imaging on the subject. In one specific aspect, the method comprises performing an MRI on the subject.

[00105] The disclosure further provides a method of therapeutic imaging of a subject in need thereof. The method comprises treating a subject's disease, for example atherosclerosis, by administering to the subject the lipid nanoparticle complex, or composition comprising the lipid nanoparticle complex and imaging a body part or a tissue of the subject in vivo and/or in vitro. Therapeutic imaging includes the magnetic resonance-guided focused ultrasound treatment and/or drug delivery. Such methods can include detecting the location of diseased area through administering to the subject the lipid nanoparticle complex, or

composition thereof, followed by magnetic resonance imaging, and optionally ablating the diseased area using an integrated ultrasound.

[00106] The disclosure further encompasses a method of target-specific imaging. The method comprises administering to a subject the lipid nanoparticle complex, or a composition thereof, and performing an imaging on the subject. The imaging comprise MRI. In such aspects, the target comprises atherosclerotic lesion/plaque, or atherosclerotic tissue.

[00107] Further provided herein is a method of imaging an atherosclerotic lesion/plaque in a subject. The method comprises administering to a subject the lipid nanoparticle complex, or a composition thereof, and performing an imaging on the subject. The imaging may comprise MRI. The atherosclerotic lesion may be a stable atherosclerotic plaque, stable atherosclerotic lesion, vulnerable atherosclerotic plaque and vulnerable atherosclerotic lesion. The atherosclerosis may be associated with plaque rupture, plaque erosion, acute coronary syndrome, stroke, transient ischemia attack, heart attack, angina, unstable angina, thrombosis, myocardial infarction, ischemic heart disease, peripheral artery disease, or transplantation-induced sclerosis.

[00108] The disclosure further comprises a method of diagnosing atherosclerotic lesion/plaque in a subject comprising: administering to the subject a lipid nanoparticle complex or a composition thereof and performing an imaging. The imaging may comprise MRI.

[00109] In a specific aspect, a method of detecting early atherosclerotic lesion/plaque is further provided. The method comprises administering to a subject the lipid nanoparticle complex, or a composition thereof, and performing an imaging on the subject. The imaging may comprise MRI.

[00110] Also provided is a method of imaging to plaque development. The method comprises administering to a subject the lipid nanoparticle complex, or a composition thereof, and performing an imaging on the subject. The imaging may comprise MRI. The disclosed method can be used to image lesion/plaque in areas of branching and high curvature, for example, carotid bifurcations and aortic root.

[00111] The lipid nanoparticle complex, or composition thereof can be used for staging an atherosclerotic lesion/plaque. The method comprises administering to a subject the lipid nanoparticle complex, or a composition thereof, and performing an imaging on the subject. The imaging may comprise MRI. The method further comprises providing the stage of

atherosclerotic lesion/plaque. The stages of atherosclerotic lesion/plaque can include, early, middle, or late stages. Alternatively, the stages can include, but not limited to early fatty streak phase, early fibro atheroma phase, fibrous plaque, complicated plaque, plaque rupture, necrotic core, and complete block of artery.

[00112] The lipid nanoparticle complex, or composition comprising the lipid nanoparticle complex can be administered at an amount that enhances contrast of an image of a body part or tissue of the subject. In one aspect, lipid nanoparticle complex, or composition comprising the lipid nanoparticle complex can be administered at a diagnostically effective amount, sufficient to enhance the contrast effect, to a degree necessary for the diagnosis of the subject.

[00113] The lipid nanoparticle complex, or composition thereof is administered to the subject for example, intravenously at a dose of about 0.5 to 20 mg/kg, optionally repeated as necessary with a waiting time between 12 and 144 hours or longer. The dose may be about 0.1 mg/kg, about 0.2 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 1.2 mg/kg, about 1.5 mg/kg, about 2.0 mg/kg, or about 2.5 mg/kg. In another aspect, the dose may be about 100 mg/kg, about 50 mg/kg, about 30 mg/kg, about 20 mg/kg, about 18 mg/kg, about 16 mg/kg, about 15 mg/kg, about 14 mg/kg, about 12 mg/kg, about 10 mg/kg, about 9 mg/kg, about 8 mg/kg, about 7 mg/kg, about 6 mg/kg, or about 5 mg/kg. In certain aspects, any of the doses may be repeated as necessary. The repeat dose may be the same or different from the previous dose. In certain aspects, each repeat has about the same dose. Optionally, the waiting time between the repeats may be between 12 (0.5 day) and 144 hours (12 days) or longer, or between 1-10 days, or between 2-10 days, between 3-10 days, between 4-10 days, between 5-10 days. In certain aspects, the waiting time between the repeats may be at least about 1, 2, 3, 4, 5, 6, or 7 days. In some aspects, the waiting time between the repeats may be up to 20, 18, 16, 15, 14, 12, 10, 9, 8, 7, 6, or 5 days. The intravenous dose for MRI can vary based in part on the route of administration, the magnetic field strength, and the structures that need to be separately visualized, and can be optimized by one skilled in the art using routine experimentation.

[00114] Alternatively, the lipid nanoparticle complex, or composition thereof is administered interstitially for nodal enhancement at a dose of about 0.01-2 mg/kg, with stimulation of lymphatic uptake as feasible, and, optionally repeated as necessary with a waiting

time of between 30 min-14 days. In another aspect, lipid nanoparticle complex, or composition thereof is administered intracavitarily at a dose of about 0.05-2 mg/kg in an appropriate suspension and waiting 30 min-14 days before imaging.

[00115] Imaging, for example MRI, can be performed by a professional or an imaging technician. The settings for imaging can be optimized based on the judgement of the professional/technician and can vary depending upon, among many things, the subject's condition, tissue to be images, the imaging machine or technique used. Skilled artisans will be able to optimize the settings without undue experimentation. For example, the skilled artisan can optimize suitable magnetic fields, which can be used on a subject. Exemplary magnetic field strengths range from about 0.01 Tesla to about 7 Tesla, about 0.02 Tesla to about 6 Tesla, about 0.03 Tesla to about 5 Tesla, about 0.04 Tesla to about 4 Tesla, about 0.05 Tesla to about 3 Tesla, about 0.05 Tesla to about 2 Tesla, about 0.05 Tesla to about 1.5 Tesla, about 0.07 Tesla to about 1.2 Tesla, about 0.1 Tesla to about 1.1 Tesla, about 0.2 Tesla to about 1.05 Tesla, about 0.3 Tesla to about 1 Tesla, about 0.4 Tesla to about 1 Tesla about 0.5 Tesla to 1 Tesla, about 0.6 Tesla to 1 about Tesla, about 0.7 Tesla to 1 Tesla, and about 1 Tesla.

[00116] Images obtained can be analyzed for by a physician or the technician to provide specific indication and provide an assessment or diagnosis of the condition. The physician can further provide or prescribe a treatment based on the identified condition. Images, for example, images of the atherosclerotic lesion/plaque can be taken before and after treatments, or a healthy subject, and can be visually compared to read differences in the condition. Alternatively, the images may be analyzed by a computer, using a computer-based grading using a computational algorithm that have been previously validated for assessment of the lesion/plaque condition.

[00117] In one aspect, the lipid nanoparticle complex, or composition thereof enhances the contrast of an image by at least 10%, compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. For example, the lipid nanoparticle complex, or composition thereof can enhance the contrast of an image by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane.

[00118] In another aspect, the lipid nanoparticle complex, or composition thereof enhances T2 relaxivity by at least 10%, compared to nanoparticles not cloaked, encapsulated or

coated with a plasma membrane. For example, the lipid nanoparticle complex, or composition thereof can enhance T2 relaxivity by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. In a specific aspect, the lipid nanoparticle complex, or composition thereof can enhance T2 relaxivity by at least by 60%.

[00119] In yet another aspect, the lipid nanoparticle complex, or composition thereof decreases T2* signal intensity by at least 10%, compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. For example, the lipid nanoparticle complex, or composition thereof can decrease T2* signal intensity by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% compared to nanoparticles not cloaked, encapsulated or coated with a plasma membrane. In a specific aspect, the lipid nanoparticle complex, or composition thereof can decrease T2* signal intensity by at least by 90%.

[00120] The methods provided herein can further comprise providing a suitable treatment to the subject. If the disclosed methods detect or diagnose the subject as having atherosclerotic lesion/plaque, suitable treatment may be administered to the subject for treatment of the lesion/plaque, based on the severity of the condition. Non-limiting examples of treatment can include stent insertion, angioplasty, or administering a streptokinase, tissue plasminogen activator, plasmin, urokinase, a tissue factor protease inhibitor, a nematode-extracted anticoagulant protein, a metalloproteinase inhibitor, an anti-inflammatory agent(s), a statin, HDL (e.g., the major protein of HDL, apo A1 or a peptide component of apo A1 that has therapeutic activity similar to that of HDL; mutant apo A1, such as apo A1 Milano or other mutant form that has similar therapeutic activity). Other specific examples of therapeutic agents include, but are not limited to the following: (a) agents that reduce lipid levels in atherosclerotic lesions, such as an HMG- CoA reductase inhibitor, a thyromimetic, a fibrate, or an agonist of peroxisome proliferator- activated receptors (PPAR); (b) agents that reduce an oxidative process in a mammal such as cytokine-stimulated cyclohydrolase-1 (GTPCH-I) or haptoglobin; (c) agents that modulate expression of an endothelial cell receptor, an endothelial cell adhesion molecule, an endothelial cell integral, a smooth muscle cell receptor, a smooth muscle cell adhesion molecule or a smooth muscle cell integrin; (d) agents that modulate the proliferation of an endothelial cell or a smooth muscle cell in a mammalian blood vessel; (e) agents that modulate an inflammation

associated receptor (or the ligand of such receptor, or the signaling pathway of such a receptor); or inflammation associated transcription factors, such as chemokine receptors, tissue factor (TF); RAGE receptors, toll-like receptors, angiotensin receptors, a TGF receptor, interleukin receptors, a TNF receptor, an IFN γ receptor, a metabotropic glutamate 8 (mGlu8) receptor, receptors that can activate NF- κ b; e) agents that modulate an inflammation associated receptor (or the ligand of such receptor, or the signaling pathway of such a receptor); or an inflammation associated transcription factor selected from the group consisting of a chemokine receptor, tissue factor (TF); a RAGE receptor, a toll-like receptor, an angiotensin receptor, a TGF receptor, an interleukin receptor, a TNF receptor, an IFN γ receptor, a metabotropic glutamate 8 (mGlu8) receptor, a receptor that can activate NF- κ b; CXCL9 (Mig); CXCL10 (IP-10); CXCL11 (I-TAC); CXCL12 (SDF-1, and its receptor CXCR4); CCL17 (TARC); CCL22 (MDC); CCL3 (MIP-1 alpha); CCL5 (RANTES); CCL7 (MCP-3); CXCL1 (growth-regulated oncogene-alpha); CXCL5 (ENA-78); CXCL8 (IL-8); fractalkine (FKN; and its receptor, CX3CR1); HSP60; toll-like receptors; metabotropic glutamate receptors (mGluRs; and its ligands such as (S)- 2-amino-4-phosphonobutanoic acid and (S)-3,4-dicarboxyphenylglycine); c-Fos, IL-6, IL- 8, MCP-1, IFN- γ , and TNF- α ; and adhesion molecules such as VCAM-1 and ICAM-1; (f) agents that modulate proliferation, apoptosis or necrosis of endothelial cells, vascular smooth muscle cells, lymphocytes, monocytes, or neutrophils; (g) agents that modulate production, degradation, or crosslinking of an extracellular matrix protein such as collagen, a elastin, or a proteoglycan; (h) agents that modulate activation, secretion or lipid loading of a cell within a mammalian blood vessel; (i) agents that modulate activation or proliferation of a dendritic cell or monocyte/macrophage cell within a mammalian blood vessel; (j) agents that modulate activation or adhesion of a platelet at a mammalian blood vessel wall or could otherwise inhibit clot formation, such as activated protein C (APC); and (k) an agent consisting of a nucleic acid that encodes a protein therapeutic agent, wherein the protein therapeutic agent has an in vivo activity that is beneficial to a mammal suffering from an atherosclerotic lesion.

[00121] Therapeutic agents can include genes or nucleic acids that encode proteins or antisense RNAs that inhibit inflammatory events at the sites of atherosclerosis lesion progression or at sites of vulnerable atherosclerotic lesions. Such genes or nucleic acids include the dominant-negative form or soluble forms of the chemokine receptors (any of CCR, CXCR, or CX3CR) and adhesion molecules such as VCAM-1 and ICAM-1. Likewise, dominant-negative

forms or soluble forms of toll-like receptors (e.g., TLR-I, TLR-2, TLR-3, TLR-4 or TLR-5) maybe therapeutic agents of the invention. As another example, therapeutic agents include genes or nucleic acids that encode proteins or antisense RNAs that inhibit foam cell formation and thus retard progression and/or stimulate regression of atherosclerotic lesions. Such genes or nucleic acids can, for example, encode secreted "decoys" or mutants of macrophage scavenger receptors MSR (sMSR).

[00122] The treatment disclosed herein may result in healing at the site of atherosclerosis, especially in plaques that are vulnerable to rupturing and to producing thrombosis, unstable angina, myocardial infarction or stroke.

[00123] The methods disclosed herein be further combined with other methods of assessment and/or treatments, as determined by the practitioner responsible. The selection of any combination of assessment and/or treatment to be administered to a subject can be determined by physical and physiological factors such as severity of condition, the type of disease being treated, previous or concurrent therapeutic interventions, and idiopathy of the subject. The practitioner responsible will, in any event, determine the appropriate course of action for the individual subject.

f. Kits

[00124] The present disclosure provides a kit comprising lipid nanoparticle complex, or a composition thereof for use with the methods of the disclosure. The kit may comprise a composition comprising lipid nanoparticle complex, and instructions for administering the lipid nanoparticle complex to a subject in need thereof and/or imaging using the lipid nanoparticle complex. In some aspects, the kit can further comprise a sterile, physiologically acceptable carrier, buffer or other diluent. The kit provided herein generally include instructions for carrying out the methods. Instructions included in the kit may be affixed to packaging material or may be included as a package insert. While the instructions are typically written or printed materials, they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this disclosure. Such media include, but are not limited to, electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. As used herein, the term "instructions" may include the address of an internet site that provides the instructions.

[00125] The disclosed kit may have a single container that contains the disclosed lipid nanoparticle complex with or without any additional components, or they may have distinct containers for each desired agent.

[00126] When the components of the kit are provided in one or more liquid solutions, the liquid solution is preferably an aqueous solution, with a sterile aqueous solution being particularly preferred. However, the components of the kit may be provided as dried powder(s). When reagents or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container.

[00127] The containers of the kit will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which the disclosed lipid nanoparticle complex, and any other desired agent, may be placed and, preferably, suitably aliquoted. Where separate components are included, the kit will also generally contain a second vial or other container into which these are placed, enabling the administration of separated designed doses. The kits may also comprise a second/third container means for containing a sterile, pharmaceutically acceptable buffer or other diluent.

[00128] The kit may also contain a means by which to administer the disclosed composition to an animal or patient, e.g., one or more needles or syringes, or even an eye dropper, pipette, or other such like apparatus, from which the formulation may be injected into the animal or applied to a diseased area of the body. The kit of the present disclosure will also typically include a means for containing the vials, or such like, and other component, in close confinement for commercial sale, such as, e.g., injection or blow-molded plastic containers into which the desired vials and other apparatus are placed and retained.

[00129] Having described several aspects, it will be recognized by those skilled in the art that various modifications, alternative constructions, and equivalents may be used without departing from the spirit of the present disclosure. Additionally, a number of well-known processes and elements have not been described in order to avoid unnecessarily obscuring the present disclosure. Accordingly, this description should not be taken as limiting the scope of the present disclosure.

[00130] Those skilled in the art will appreciate that the presently disclosed aspects teach by way of example and not by limitation. Therefore, the matter contained in this

description or shown in the accompanying drawings should be interpreted as illustrative and not in a limiting sense. The following claims are intended to cover all generic and specific features described herein, as well as all statements of the scope of the method and assemblies, which, as a matter of language, might be said to fall there between.

EXAMPLES

[00131] All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the present disclosure pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

[00132] The publications discussed throughout are provided solely for their disclosure before the filing date of the present application. Nothing herein is to be construed as an admission that the disclosure is not entitled to antedate such disclosure by virtue of prior invention.

[00133] It is understood that the examples and aspects described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

[00134] Unless specified, reagents employed in the examples are commercially available or can be prepared using commercially available instrumentation, methods, or reagents known in the art. The examples illustrate various aspects of the disclosure and practice of the methods of the disclosure. The examples are not intended to provide an exhaustive description of the many different aspects of the disclosure. Thus, although the disclosure has been described in some detail by way of illustration and example for purposes of clarity of understanding, those of ordinary skill in the art will realize readily that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

Materials and Methods

Isolation of mouse monocyte membrane vesicles

[00135] Large bones such as the femur, tibia, and humerus were harvested from C57BL/6J mice. The bone marrow cells (BMCs) were flushed out from the bones with PBS, filtered, and differentiated in RPMI 1640 medium containing 10% FBS, 1% penicillin-

streptomycin, 1% L-glutamate, 1% sodium pyruvate, and 20 ng/mL M-CSF. Monocytes were collected after 5 days and magnetically sorted using MojoSort™ Mouse Monocyte Isolation Kit (BioLegend #480154). Monocyte membrane vesicles were isolated from these cells through a Minute™ Plasma Membrane Protein Isolation and kit (Invent Biotechnologies). The yield of monocyte membrane vesicles was quantified based on the total membrane protein content, as measured by the Bradford protein assay.

Formulation and characterization of MoNP-SPION

[00136] NP-SPION was synthesized through a double emulsion-solvent evaporation method. In this process, 10 mg of PLGA (Resomer® RG 503H, Sigma Aldrich) was dissolved in dichloromethane (DCM, Sigma Aldrich). Subsequently, 1mg of 15 nm SPIONs (iron oxide (II, III) nanoparticles, Sigma Aldrich), resuspended in pure water, was slowly added to the PLGA solution and dispersed with a probe sonicator (Qsonica) to create the initial water-in-oil (W/O) emulsion. The W/O emulsion was then added dropwise to a 2% polyvinyl-alcohol (PVA, Arcos Organics) solution and further sonicated to form the W/O/W emulsion, followed by addition of a 0.5% PVA solution. After solvent evaporation, the mixture was washed and centrifuged to collect NP-SPION. To prepare MoNP-SPION, Mo membrane vesicles and NP-SPION were mixed together in a membrane proteins-to-NP-SPION weight ratio of 1:10 and submerged in an ultrasonic bath for 5 minutes. The hydrodynamic size, PDI, and zeta potential of resulting MoNP-SPION were measured using DLS (Zetasizer, Malvern Panalytical). The morphology of MoNP-SPION and NP-SPION was examined after staining with 1% uranyl acetate using TEM (Talos L120C) at ASU Eyring Materials Center. The relaxivity was measured through inversion recovery fast spin echo T1 map (TE: 11; TR: 6000; FOV: 40; matrix: 64x64) and multi-echo multi slice T2 map (TE: 10; TR: 2500; FOV: 40; matrix: 64x64) sequences on the MRS*DRYMAG 9.4T MRI (MR Solutions). Relaxometry analysis was conducted using the Bayesian Analysis Toolbox. To determine encapsulation efficiency (EE) and loading capacity (LC), an aqueous iron assay was performed as described. Briefly, NP-SPION were lyophilized and dissolved in 20% hydrochloric acid (HCl, Arcos Organics, 42379) to form ferric chloride. 5% potassium ferrocyanide (EMS, 25154-5) was added to the ferric chloride solution in a 1:1 (v/v) ratio and the absorbance was measured using a plate reader (BioTek). The absorbance values were correlated to concentrations of iron based on a known iron standard curve. EE and LE calculations are as follows:

$$EE(\%) = \frac{\text{Measured Mass (SPION)}}{\text{Starting Mass (SPION)}} * 100\%$$

$$LC(\%) = \frac{\text{Measured Mass (SPION)}}{\text{Starting Mass (SPION)} + \text{Starting Mass (PLGA)}} * 100\%$$

Western blot analysis of membrane proteins

[00137] Ten micrograms of total proteins isolated from the fraction were separated using SDS-PAGE and transferred onto a nitrocellulose membrane. The membrane was then blocked with 5% BSA and incubated with the primary antibodies at 4°C overnight. The following day, the blots were incubated with the secondary antibodies. The following primary antibodies used, all diluted 1:1000 from the stocks, were: CD11b (Cell Signaling, 17800), Na⁺/K⁺ ATPase (Cell Signaling, 3010), TLR4 (BioLegend, 145401), and GAPDH (Cell Signaling, 2118). The blots were developed using a chemiluminescence kit (Pierce) and imaged with the Analytik Jena bioimaging system

In vitro cellular uptake

[00138] Human umbilical vein endothelial cells (HUVEC, ATCC), RAW264.7 macrophages (ATCC), and BMC-derived monocytes were maintained in their respective growth medium under standard cell culture conditions (37°C, 5% CO₂, 100% humidity). To assess cellular uptake, HUVEC, RAW264.7 cells, and monocytes were incubated with MoNP-SPION, NP-SPION, or SPION for 30 mins at 10 µg Fe/ml. Cells were then stained with Prussian Blue and nuclear fast red staining (Thermo Fisher, J61010) and imaged with colored brightfield microscopy (BioTek). In parallel, cells that underwent the same NP incubation were collected into a 1% low-melt agarose (Sigma-Aldrich, A9414), placed in a custom microfuge tube coil mount, and imaged on the MRS*DRY MAG 9.4T MRI using a T2*-weighted sequence (TE: 12.8; TR: 833; α: 60; FOV: 40; matrix: 256x256). Iron content was directly measured in each culture measured by digesting in 20% HCl for subsequent aqueous iron assays. To measure the NP phagocytosis by phagocytes in blood, MoNP-SPION, NP-SPION, or SPION were incubated with aliquots of mouse whole blood at a concentration of 100 µg Fe/ml. Plasma was then separated through low-speed centrifugation and collected for iron content analysis.

Animal experiments

[00139] All animal experiments performed in this study have been approved by the Institutional Animal Care and Use Committee (IACUC) of Arizona State University. C57BL/6J inbred mice were used for BMC collection and source of monocytes. Apolipoprotein E-deficient

mice (ApoE^{-/-}, originally purchased from The Jackson Laboratory) of either sex, aged 8-12 weeks old, were subjected to partial carotid ligation (PL) procedure or 12-weeks of HFD (Envigo, TD.88137) to induce acute arterial inflammation and plaque formation. Both models are well-established and widely documented in the literature for study atherosclerosis. ApoE^{-/-} mice that underwent PL procedure 10 days prior were given retro-orbital (RO) injections of MoNP-SPION, NP-SPION, SPION, or saline at a concentration of 5 mg Fe/kg. The mice were euthanized 3 hours post-administration through CO₂ inhalation, followed by the isolation of the arterial tissues. The tissues were embedded in low-melt agarose gel and imaged on the MRS*DRYMAG MRI using a T2*-weighted sequence (TE: 12.8; TR: 833; α : 60; FOV: 40; matrix: 256 x 256). For live animal imaging, ApoE^{-/-} mice maintained on HFD for 12 weeks were anesthetized, secured in a cradle, and scanned pre- and post-administration of 5 mg Fe/kg MoNP-SPION, NP-SPION, SPION, or saline, on the Bruker BioSpec 7T MRI, with MR gating systems (SA Instruments) at ASU-Barrow Center for Preclinical Imaging at Barrow Neurological Institute. All scans were performed with cardiac and respiratory gating, monitored using PC-SAM. The aortic root was identified approximately 5 mm above the base of the heart, and the carotid bifurcation roughly 16 mm above. After NP administration, post-injection imaging was conducted to locate and align the same anatomical regions with the pre-injection scans. MR imaging was conducted 2-hour post injection. Mice remained secured in the cradle throughout the entire imaging process to maintain anatomical positioning. Three imaging acquisition sequences were used: T1 Fast Low Angle Shot (FLASH) (TE: 1.7; TR: 128.3; α : 40; FOV: 30; matrix: 192 x 192), Time of Flight (ToF) FLASH bright blood (TE: 3.4; TR: 7; α : 10; FOV: 30; matrix: 256 x 256), and T2* FLASH (TE: 3.2; TR: 600; α : 50; FOV: 30; matrix: 256 x 256). Following imaging, mice were euthanized for tissue collection and histological analysis.

[00140] Circulation and toxicity studies were conducted with C57BL/6J mice, which were given RO injections of MoNP-SPION, SPION, or saline. Blood samples were drawn through submandibular cheek puncture at 1h, 2h, and 24h post-injection for iron content analysis. Mice were euthanized after 24h where blood was collected along with major organs (heart, liver, lung, kidney, spleen) for H&E staining. Serum collected from whole blood samples was then sent for metabolic panel analysis (Vetek Labs).

Image analysis

[00141] Throughout the study, ImageJ was used for analyzing the microscopic and MRI images. For quantifying the contrast change in the LCA and RCA of PL-operated ApoE^{-/-} mice, intensity thresholds were adjusted to identify the arterial tissues outside of the shadowing artifact caused by the ends of the MRI coil. Once the appropriate regions were identified, these thresholding parameters were used to generate an ROI that was used to measure the signal intensities of the tissue in both the LCA and RCA. For live animal imaging study, the aortic root regions were identified from the surrounding image using image thresholding, and bright blood images were employed to determine the lumen area. Contrast changes in the wall of aortic root were calculated by subtracting the lumen area from the entire aortic region. For the carotid bifurcation, given the minimal distortion from blood flow compared to the overall signal reduction by MoNP-SPION, the contrast change of the entire cross-sectional area, including the lumen, was measured.

Histology analysis

[00142] Tissues collected were embedded within OCT compound and flash frozen to prepare for cryosectioning. All tissues were sectioned at a thickness of 10 μ m. Prior to staining procedures, cryosections were rinsed with PBS to remove residual OCT. For Prussian Blue staining, 5% potassium ferrocyanide was added to 20% hydrochloric acid at a 1:1 (v/v) ratio. Sections were stained with the Prussian Blue solution for 30 minutes before counter staining with nuclear fast red for 1 minute. H&E-stained sections were dipped in hematoxylin for 30 seconds and counter stained with eosin for 2 minutes. All staining procedures were followed by serial dehydration with 95% and 100% ethyl alcohol and xylene prior to the application of mounting media and coverslip.

Statistical analysis

[00143] All graphical data are presented as mean $\pm$ standard deviation (SD), and statistical analysis from at least three biological repeat experiments were performed using GraphPad Prism 10.1.2. A one-way ANOVA was performed for the comparison of multiple groups followed by Tukey's multiple comparisons post-hoc test. For comparison between two groups, a two tailed unpaired t-test was used.

Example 1: MoNP-SPION demonstrates an enhanced T2 relaxivity

[00144] To achieve precise MR imaging of atherosclerosis, the first step involves formulating SPION-loaded NP cores (NP-SPIONs) using a modified double emulsion method, in which 15nm SPIONs were dispersed in the aqueous phase and encapsulated within a poly(lactic-co-glycolic acid) (PLGA) shell. The resulting NP-SPIONs were then fully cloaked within isolated mouse monocyte membranes, as previously described, yielding MoNP-SPIONs (**FIG. 1A**). A Prussian blue-based colorimetric assay indicated that approximately 70.1% of the input SPIONs were successfully encapsulated into NP cores, achieving a loading efficiency of about 8.5% (**Table 1**). The physicochemical characteristics of MoNP-SPIONs, NP-SPIONs, and monocyte membrane vesicles were measured using dynamic light scattering (DLS), transmission electron microscopy (TEM), Western blot, and MR relaxometry. The DLS data showed that MoNP-SPIONs exhibited a uniform size distribution with a low polydispersity index (PDI) of approximately 0.2 and a mean hydrodynamic diameter of 271 ± 49 nm, slightly larger than uncoated NP-SPIONs (229 ± 47 nm) (**FIG. 1B**). The ζ -potential of MoNP-SPIONs was -22.6 ± 2.55 mV, much closer to the surface charge of monocyte membrane vesicles than NP-SPIONs, indicating effective membrane cloaking (**FIG. 1C**). TEM images confirmed the successful encapsulation of SPIONs within a PLGA shell and the subsequent cloaking of NP-SPIONs with monocyte membranes to form MoNP-SPIONs (**FIG. 1D**). Western blot analysis showed that monocyte membrane proteins, including CD11b, TLR4, and Na^+/K^+ -ATPase, were retained in MoNP-SPIONs, while the cytoplasmic marker GAPDH was not detected (**FIG. 1E**). MR phantom imaging demonstrated hypointense contrast changes in phantoms dispersed with MoNP-SPIONs, NP-SPIONs, or SPIONs as their concentrations increased (**FIG. 1F**). Further relaxometry analysis revealed that MoNP-SPIONs had a T2 relaxivity (r_2) of 400 ± 23.7 $\text{mM}^{-1}\text{s}^{-1}$, a 66% enhancement compared to 240 ± 23.1 $\text{mM}^{-1}\text{s}^{-1}$ measured for SPIONs (**FIG. 1G** and **Table 1**). Additionally, none of these samples exhibited significant T1 relaxivity (r_1) (**FIG. 2A-2B**). These results demonstrate that we can successfully incorporate SPIONs into our biomimetic MoNP delivery platform, resulting in MoNP-SPIONs with significantly enhanced T2 relaxivity compared to the constituent SPIONs.

Table 1: Characterization of NP

	SPION	Mo	NP-SPION	MoNP-SPION
Size (nm)	-	320.16 ± 86.96	229.86 ± 47.16	271.16 ± 49.19
Zeta (mV)	-	-18.66 ± 1.10	-36.9 ± 2.18	-22.6 ± 2.55
PDI	-	0.31 ± 0.02	0.19 ± 0.04	0.20 ± 0.04

r1 (mM⁻¹*s⁻¹)	0.25±0.19	-	0.19±0.41	0.14±0.22
r2 (mM⁻¹*s⁻¹)	240.3±23.1	-	434.0±24.6	400.0±23.7
E.E. (%)	-	-	70.126±7.81	-
L.C. (%)	-	-	8.564±2.59	-

Example 2: MoNP-SPION enables preferential uptake by inflamed endothelium while avoiding phagocytes

[00145] Persistent endothelial activation leads to constant infiltration of circulating monocytes into the vessel wall, significantly contributing to plaque development; however, it also provides a pathway for MoNP-SPIONs to specifically target and image atherosclerotic vessels. To demonstrate the selective targeting of MoNP-SPIONs to ECs in atherosclerotic environment, *in vitro* studies were conducted in which MoNP-SPIONs, NP-SPIONs, or SPIONs were incubated with human umbilical cord ECs (HUVECs) pretreated with TNF α , followed by iron staining and measurements and MR phantom imaging. Prussian Blue staining revealed that MoNP-SPION treatment results in a substantially higher accumulation of iron in TNF α -activated ECs, compared to cells incubated with NP-SPIONs or SPIONs (**FIG. 3A-3B**). Quantitative analysis of iron content confirmed this finding, showing a 2.98±0.25-fold increase was measured for MoNP-SPION group in TNF α -activated ECs when normalized to the saline control, whereas NP-SPION and SPION had only 1.42±0.25-fold and 1.51±0.32 increases, respectively. T2*-weighted MR imaging of EC-loaded phantoms further illustrated that a hypointense contrast change was observable only in TNF α -activated ECs incubated with MoNP-SPIONs, but not in other groups (**FIG. 3C**). Quantification of the phantom images revealed that incubation of TNF α -treated ECs with MoNP-SPIONs induced a 0.76±0.06-fold reduction in T2* signal intensity (**FIG. 3D**). These data suggest that MoNP-SPIONs can actively target sites of endothelial activation, enabling hypointense MR contrast change specifically in atherosclerotic vessels.

[00146] The interactions between MR contrast agents and immune cells following intravenous (i.v.) administration are crucial in determining their fate and effectiveness for imaging. To evaluate the specificity and immune evasion capability of MoNP-SPIONs, the uptake of MoNP-SPIONs, NP-SPIONs, and SPIONs by RAW264.7 macrophages and murine bone marrow-derived monocytes were measured. The results indicated that both cultures treated

with NP-SPIONs exhibited significant Prussian Blue staining, whereas cells incubated with MoNP-SPIONs or SPIONs displayed minimal staining (**FIG. 3E**). Iron measurements in the lysates showed that NP-SPIONs were highly susceptible to phagocytosis, showing a 2.91 ± 0.22 increase in RAW264.7 and a 4.68 ± 1.05 -fold increases in primary monocytes compared to the control groups. In contrast, MoNP-SPIONs and SPIONs demonstrated significantly lower uptake (**FIG. 3F**). Consistent with these findings, MR phantom imaging showed that phagocytes treated with NP-SPIONs induced stronger hypointense signal compared to those incubated with MoNP-SPIONs or SPIONs (**FIG. 3G**). These findings were corroborated by quantification of MR images, where NP-SPIONs-treated RAW264.7 and monocytes showed a 0.77 ± 0.08 and 0.65 ± 0.12 -fold decrease in T2* signal intensity, respectively (**FIG. 3H**). To further assess the interactions of MoNP-SPIONs with phagocytes, these particles were incubated with murine whole blood and measured the residual iron levels in the plasma. In line with the findings in cultured phagocytes, the results showed that the plasma fractions of MoNP-SPION and SPION groups had significantly higher iron levels than that of the NP-SPION group (**FIG. 3I**). Together, these data suggest that MoNP-SPIONs can actively target atherosclerotic endothelium while also exhibiting an enhanced capability of phagocytic evasion compared to uncoated NP-SPIONs.

Example 3: MoNP-SPION administration facilitates targeted imaging of early atherosclerotic lesions

[00147] Next, it was sought to determine whether MoNP-SPIONs could actively target and selectively accumulate within inflamed regions of the vasculature in mice developing atherosclerosis. A partial carotid ligation (PL) procedure was performed on left carotid artery (LCA) of ApoE^{-/-} mice, which were subsequently fed a high-fat diet (HFD) to induce endothelial inflammation and lesion development within the LCA (**FIG. 4B**). Ten days after PL, mice received retro-orbital (RO) injection of MoNP-SPIONs, NP-SPIONs, SPIONs, or saline and were euthanized 3 hours later. Arterial tissues, spanning from the carotid bifurcation to the descending aorta, were harvested, embedded in agarose gel, and subjected to *ex vivo* MR imaging. The imaging results demonstrated that the administration of MoNP-SPIONs, but not NP-SPIONs, SPIONs, or saline, induced a strong hypointense T2* contrast specifically in the PL-operated LCA, with markedly less signal changes in other regions of the arterial segment (**FIG. 4C**). Quantifications of the MRI images revealed that MoNP-SPION treatment resulted in

a $20.37 \pm 3.44\%$ reduction in total signal intensity in the LCA compared to the unligated right carotid artery (RCA), while NP-SPIONs, SPIONs, and saline produced reductions of only $6.26 \pm 0.6\%$, $5.31 \pm 5.52\%$, and $2.52 \pm 2.08\%$, respectively. (**FIG. 4D**). These findings confirm that monocyte membrane cloaking facilitates the active targeting of MoNP-SPIONs to early atherosclerotic regions, inducing hypointense contrast change in MR imaging. This supports the hypothesis that MoNP-SPIONs could serve as a sensitive contrast agent for detecting the early stages of the disease.

Example 4: MoNP-SPION induces T2* contrast within the existing plaques in vivo

[00148] To Having demonstrated the effectiveness of MoNP-SPIONs in targeting early atherosclerotic lesions in the PL model and inducing visible site-specific contrast changes *ex vivo*, we proceeded to validate their capability in imaging existing plaques in live animals. In place of the PL, ApoE^{-/-} mice were maintained on a HFD for 12 weeks to induce atherosclerosis (**FIG. 5A**). This regimen typically leads to plaque development throughout the arterial tree, with more pronounced lesions occurring in areas of branching and high curvature, such as carotid bifurcations and aortic root. To test the MoNP-SPIONs' efficacy in these regions, the atherosclerotic mice were anesthetized and conducted the axial time-of-flight (ToF) bright-blood sequence to precisely localize the carotid bifurcation and aortic root. Subsequently, the T2*-weighted Fast Low Angle Shot (FLASH) sequence was employed to establish the background signal in these arterial regions. Immediately following these preparations, the mice received a RO administration of MoNP-SPIONs, NP-SPION, SPIONs, or saline and underwent a 2nd T2* FLASH scan 2-hour post-injection to capture the ensuing contrast changes in these atherosclerotic regions (**FIG. 5B**). The MR images demonstrated that MoNP-SPIONs induced noticeable T2* contrast changes in the bifurcations of LCA and RCA, as evidenced by the hypointense blooming effect observed in the post-administration images. Quantification of the bifurcation areas revealed that a significant reduction of $41.07 \pm 11.47\%$ in contrast before and after MoNP-SPIONs administration, while minimal changes were detected in mice receiving NP-SPIONs, SPIONs, or saline (**FIG. 5C**). In a similar vein, hypointense contrast changes were observed within the aortic root only in mice receiving MoNP-SPIONs, and quantification of the contrast peri-lumen regions showed a $40.05 \pm 18.2\%$ reduction in contrast, compared to $7.07 \pm 1.22\%$, $10.28 \pm 5.59\%$, $2.19 \pm 1.18\%$ of the NP-SPION, SPION, and saline groups,

respectively. (**FIG. 5D**). T1-weighted FLASH images of carotid bifurcation and aortic root were acquired to confirm that any contrast effect observed is solely a T2*-based effect resulting from iron deposition within the vasculature (**FIGs. 6A-6B**). Following imaging, the mice were swiftly euthanized, and their arterial tissues were harvested and cryo-sectioned. Prussian blue staining of arterial sections showed that MoNP-SPION treatment, but not NP-SPION, SPION, or saline groups, strongly increased the presence of iron in both the carotid bifurcations and aortic root, indicative of enhanced contrast agent delivery. (**FIG. 5E**). Together, the live animal MR imaging and subsequent histological examination of the same atherosclerotic arterial region confirm that MoNP-SPION is effective in detecting existing and active plaques in live animals. Given the monocyte-mimicking properties of MoNP-SPIONs, it could also provide insights into the functional status of the plaques.

Example 5: MoNP-SPION demonstrates minimal toxicity

[00149] MoNP-SPIONs, NP-SPIONs, or SPIONs were incubated with mouse whole blood for 30 mins at RT, and the results showed that MoNP-SPIONs and NP-SPIONs are less likely to cause hemolysis compared to standalone SPIONs (**FIG. 7A**). To further assess the safety of MoNP-SPIONs, mice were administered MoNP-SPIONs or SPIONs. Twenty-four hours post-administration, tissue samples including whole blood and vital organs were harvested for metabolic panel profiling and histopathological examination. The results indicated no significant differences in enzymes and metabolic levels representing liver and kidney functions between the MoNP-SPION- and SPION-treated groups, with all mean values within normal ranges (**FIG. 7B**). Histological examination of the organs including the heart, lung, liver, kidney, and spleen via H&E-stained sections revealed the absence of noticeable pathological injuries related to the injection of MoNP-SPIONs or SPIONs (**FIG. 7C**). These findings establish MoNP-SPION as a highly biocompatible and safe contrast agent for MR imaging, demonstrating minimal toxicity both *in vitro* and *in vivo*.

Summary of Examples

[00150] In summary, a biomimetic nano-diagnostic tool was developed that used monocyte membrane-coated nanoparticle (MoNP) platform to deliver magnetic resonance (MR) contrast agents, aimed at enhancing the imaging of atherosclerotic lesions by specifically targeting the inflamed endothelium lining the plaque surface. Specifically, superparamagnetic

iron oxide nanoparticles (SPIONs) were first encapsulated within a polymeric shell to nanoparticle encapsulated SPION (NP-SPIONs), followed by cloaking with the plasma membrane isolated from mouse monocytes, resulting in MoNP-SPIONs. Through *in vitro* assays, it was found that SPION-loaded NPs exhibit higher T2 relaxivity than free SPION counterpart and that MoNP-SPIONs are more efficient at entering inflamed ECs while reducing uptake by phagocytes, comparing to their constituent components. More importantly, the *ex vivo* and live animal MR imaging indicate that MoNP-SPIONs preferentially accumulate in regions of atherosclerosis, producing significantly stronger hypointense signals than standalone SPIONs. These findings together demonstrate that MoNP-mediated delivery of SPIONs represents a promising and more effective strategy for MR imaging of atherosclerosis.

EMBODIMENTS

[00151] The following non-limiting embodiments provide illustrative examples of the disclosure, but do not limit the scope of the disclosure.

Embodiment 1

[00152] Provided herein is a lipid nanoparticle complex comprising: (a) a superparamagnetic nanoparticle encapsulated with a polymer shell; and (b) a lipid membrane coating the polymer encapsulated superparamagnetic nanoparticle.

Embodiment 2

[00153] The lipid nanoparticle complex of Embodiment 1, wherein the lipid membrane comprises a monocyte plasma membrane.

Embodiment 3

[00154] The lipid nanoparticle complex of any one of the Embodiments 1-2, wherein the lipid membrane does not comprise a cytosolic protein.

Embodiment 4

[00155] The lipid nanoparticle complex of any one of the Embodiments 1-3, wherein the lipid membrane comprises at least one membrane protein.

Embodiment 5

[00156] The lipid nanoparticle complex of Embodiment 4, wherein the membrane protein is selected from an adhesion protein, a glycoprotein, a type I transmembrane protein, an integrin, CD11b, TLR4, and Na⁺/K⁺-ATPase.

Embodiment 6

[00157] The lipid nanoparticle complex of any one of the Embodiments 1-5, wherein the superparamagnetic nanoparticle comprises nanoparticle loaded with iron-oxide, iron cobalt, any other particle exhibiting paramagnetic properties, or a combination thereof.

Embodiment 7

[00158] The lipid nanoparticle complex of Embodiment 6, wherein the superparamagnetic nanoparticle comprises nanoparticle loaded with iron-oxide.

Embodiment 8

[00159] The lipid nanoparticle complex of any one of the Embodiments 1-7, wherein the polymer shell encapsulating the superparamagnetic nanoparticle comprises poly (lactic-co-glycolic acid) (PLGA).

Embodiment 9

[00160] The lipid nanoparticle complex of Embodiment 1, wherein the complex has an average hydrodynamic diameter of about 245 nm to about 295 nm.

Embodiment 10

[00161] The lipid nanoparticle complex of any one of the Embodiments 1-10, wherein the complex has a surface charge of about -20 to about -40 mV.

Embodiment 11

[00162] Further provided herein is a composition comprising the lipid nanoparticle complex of any one of the preceding Embodiments, and a carrier or excipient.

Embodiment 12

[00163] Provided is a method of producing a lipid nanoparticle complex comprising: (a) forming a superparamagnetic nanoparticle; (b) encapsulating the superparamagnetic nanoparticle with a polymer shell; (c) isolating a plasma membrane from a monocyte; and (d) contacting the plasma membrane of (c) with the encapsulated superparamagnetic nanoparticle (b) to form the lipid nanoparticle complex.

Embodiment 13

[00164] The method of Embodiment 12, wherein the monocyte is isolated from a bone marrow of a subject.

Embodiment 14

[00165] The method of Embodiment 12, wherein encapsulating the nanoparticle with a polymer shell comprises preparing a PLGA emulsion.

Embodiment 15

[00166] The method of Embodiment 12, wherein superparamagnetic nanoparticle comprises nanoparticle loaded with iron-oxide formed using double emulsion/solvent evaporation.

Embodiment 16

[00167] A method of imaging atherosclerotic lesion/plaque in a subject in need thereof, comprising: (a) administering to the subject a lipid nanoparticle complex of Embodiment 1 or a composition of Embodiment 11; and performing an imaging.

Embodiment 17

[00168] The method of Embodiment 16, wherein the composition is administered by injection.

Embodiment 18

[00169] The method of Embodiment 16, wherein the imaging is a magnetic resonance imaging (MRI).

Embodiment 19

[00170] The method of Embodiment 16, further comprising providing an appropriate treatment to the subject.

Embodiment 20

[00171] A contrast enhancing agent for use in MRI comprising the lipid nanoparticle complex of Embodiment 1 or a composition of Embodiment 11.

CLAIMS

What is claimed is:

1. A lipid nanoparticle complex comprising: (a) a superparamagnetic nanoparticle encapsulated with a polymer shell; and (b) a lipid membrane coating the polymer encapsulated superparamagnetic nanoparticle.
2. The lipid nanoparticle complex of claim 1, wherein the lipid membrane comprises a monocyte plasma membrane.
3. The lipid nanoparticle complex of claim 1, wherein the lipid membrane does not comprise a cytosolic protein.
4. The lipid nanoparticle complex of claim 1, wherein the lipid membrane comprises at least one membrane protein.
5. The lipid nanoparticle complex of claim 4, wherein the membrane protein is selected from an adhesion protein, a glycoprotein, a type I transmembrane protein, an integrin, CD11b, TLR4, and Na^+/K^+ -ATPase.
6. The lipid nanoparticle complex of the claim 1, wherein the superparamagnetic nanoparticle comprises nanoparticle loaded with iron-oxide, iron cobalt, any other particle exhibiting paramagnetic properties, or a combination thereof.
7. The lipid nanoparticle complex of claim 6, wherein the superparamagnetic nanoparticle comprises nanoparticle loaded with iron-oxide.
8. The lipid nanoparticle complex of the claim 1, wherein the polymer shell encapsulating the superparamagnetic nanoparticle comprises poly (lactic-co-glycolic acid) (PLGA).
9. The lipid nanoparticle complex of claim 1, wherein the complex has an average hydrodynamic diameter of about 245 nm to about 295 nm.
10. The lipid nanoparticle complex of the claim 1, wherein the complex has a surface charge of about -20 to about -40 mV.

11. A composition comprising the lipid nanoparticle complex of the claim 1, and a carrier or excipient.
12. A method of producing a lipid nanoparticle complex comprising:
 - (a) forming a superparamagnetic nanoparticle;
 - (b) encapsulating the superparamagnetic nanoparticle with a polymer shell;
 - (c) isolating a plasma membrane from a monocyte; and
 - (d) contacting the plasma membrane of (c) with the encapsulated superparamagnetic nanoparticle (b) to form the lipid nanoparticle complex.
13. The method of claim 12, wherein the monocyte is isolated from a bone marrow of a subject.
14. The method of claim 12, wherein encapsulating the nanoparticle with a polymer shell comprises preparing a PLGA emulsion.
15. The method of claim 12, wherein superparamagnetic nanoparticle comprises nanoparticle loaded with iron-oxide formed using double emulsion/solvent evaporation.
16. A method of imaging atherosclerotic lesion/plaque in a subject in need thereof, comprising:
 - (a) administering to the subject the composition of claim 11; and performing an imaging.
17. The method of claim 16, wherein the composition is administered by injection.
18. The method of claim 16, wherein the imaging is a magnetic resonance imaging (MRI).
19. The method of claim 16, further comprising providing an appropriate treatment to the subject.
20. A contrast enhancing agent for use in MRI comprising the composition of claim 11.

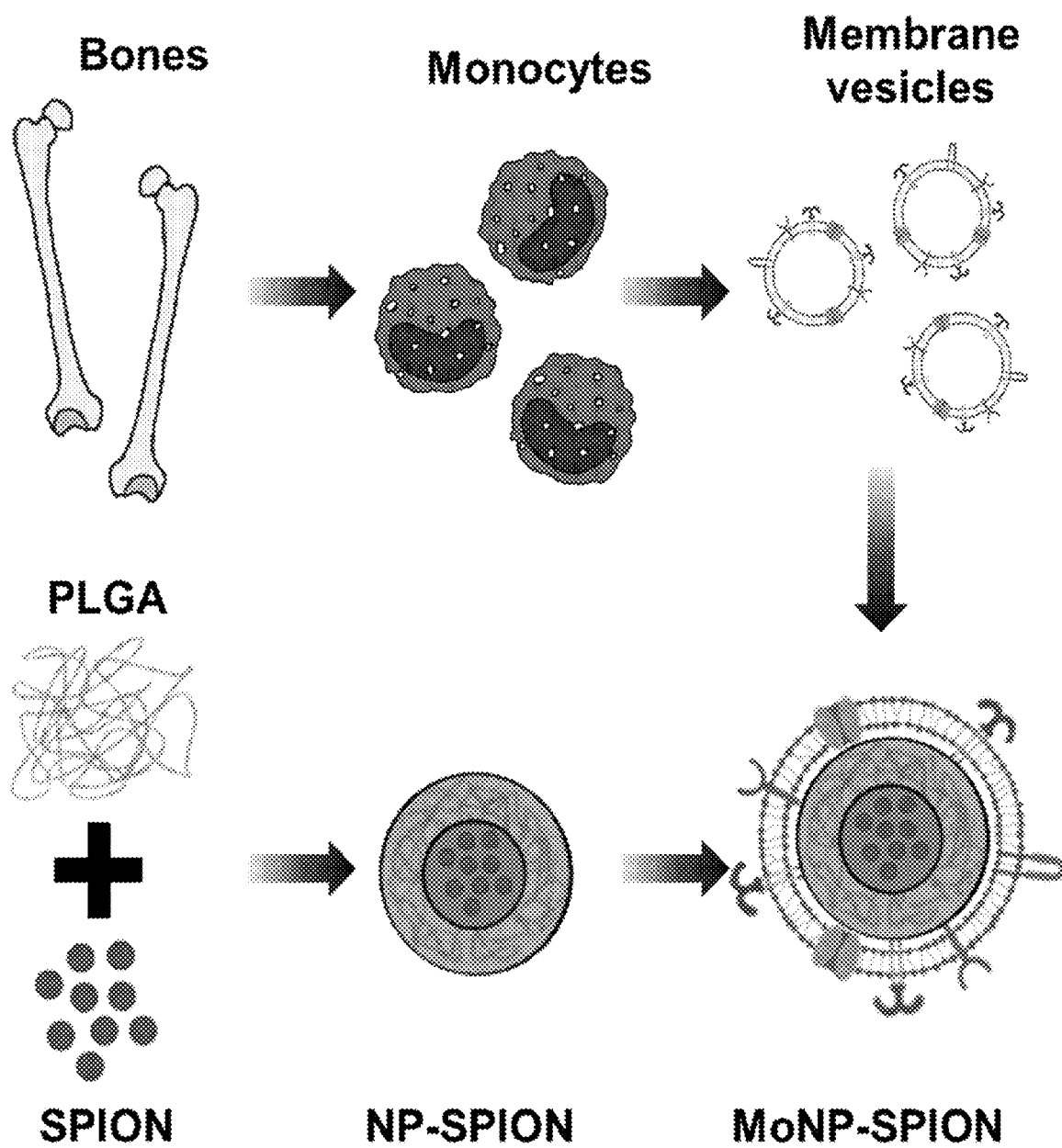


FIG. 1A

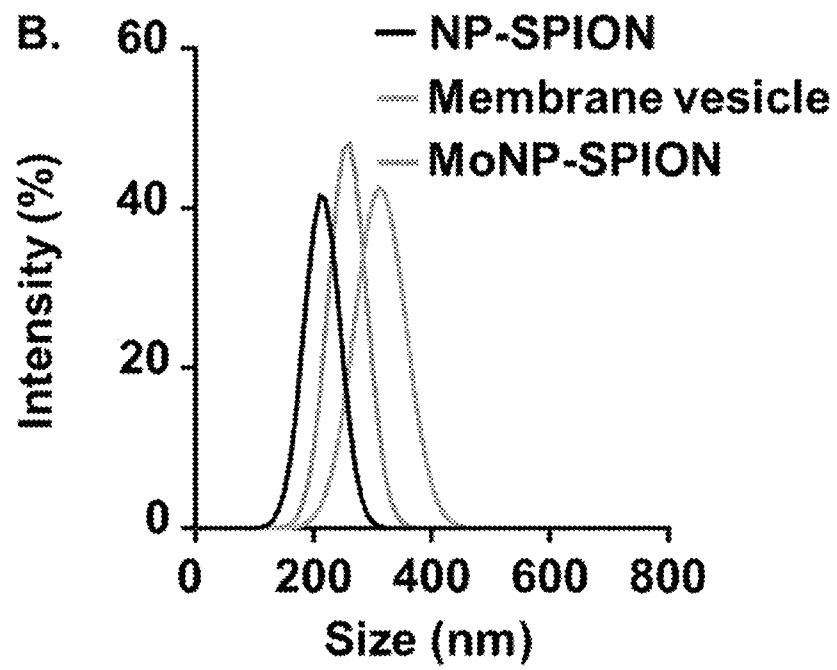


FIG. 1B

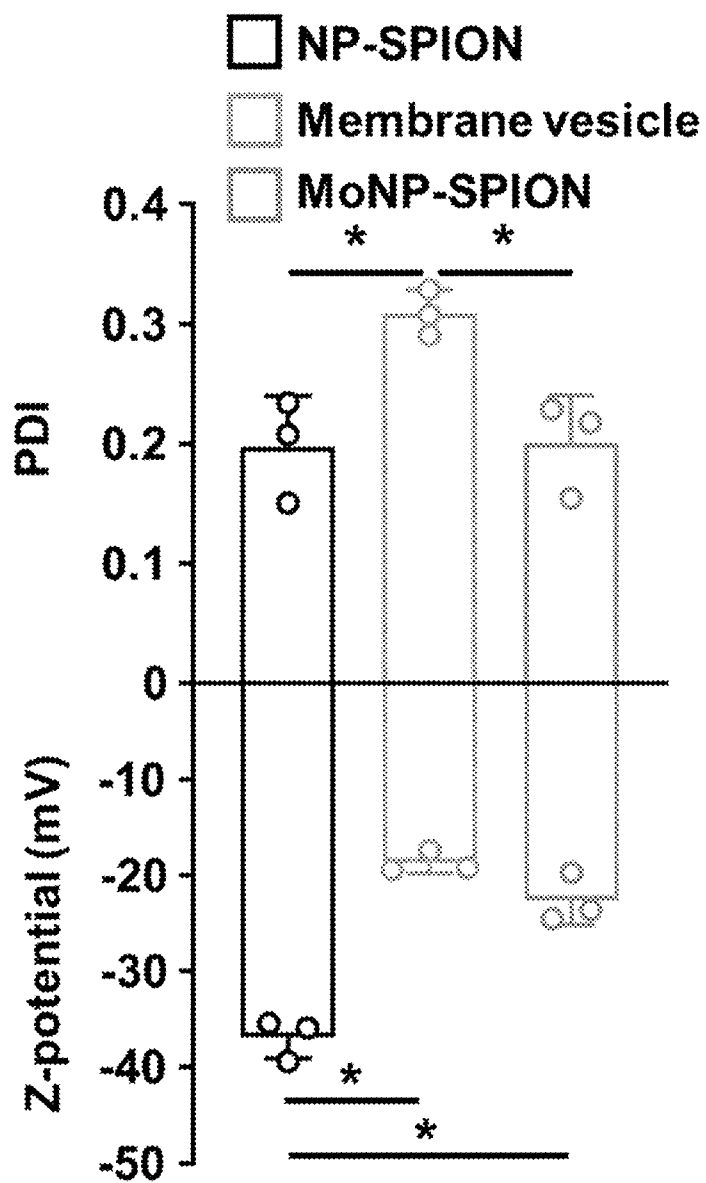


FIG. 1C

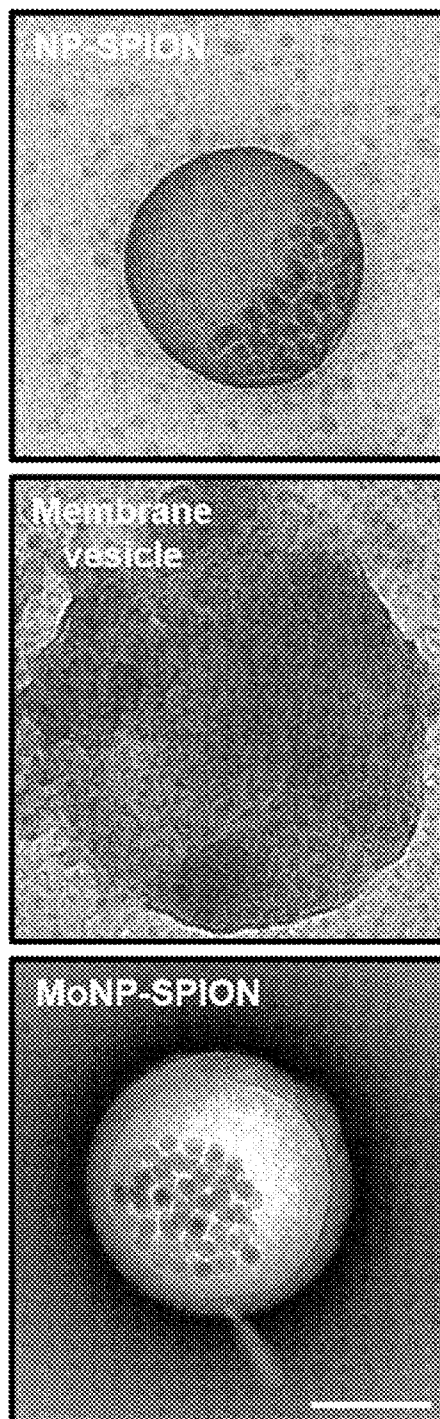


FIG. 1D

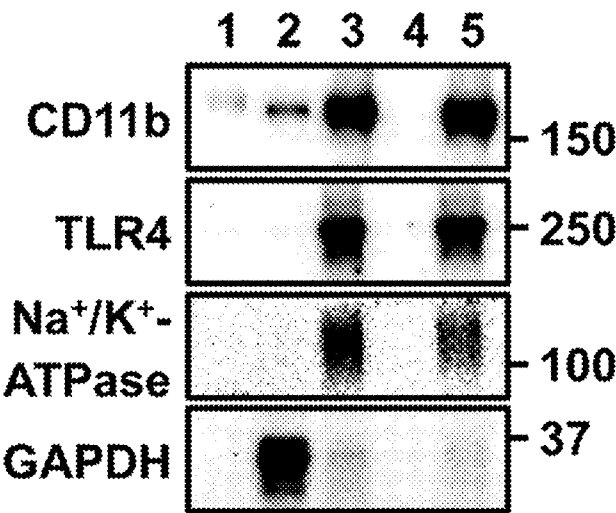


FIG. 1E

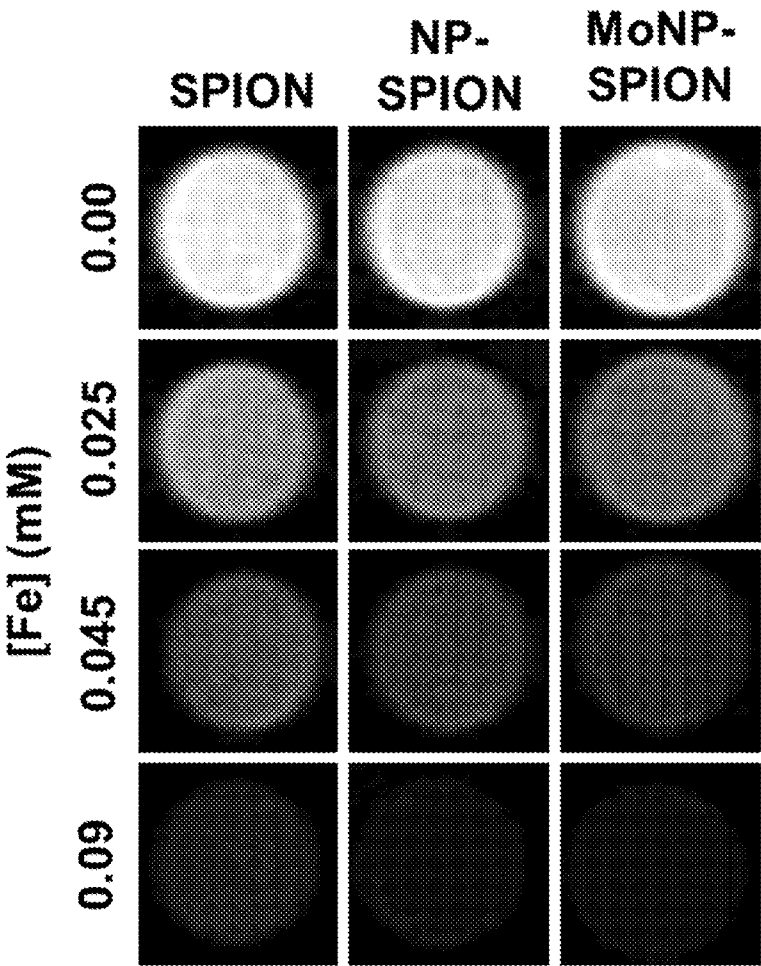


FIG. 1F

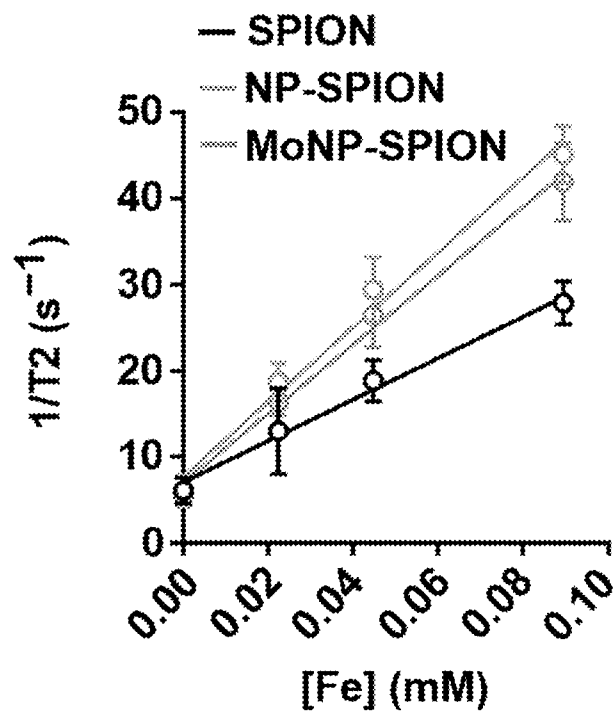


FIG. 1G

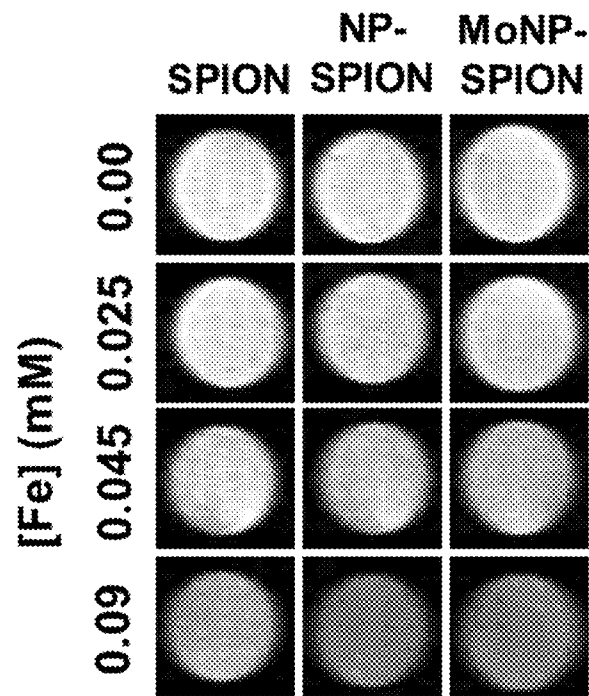


FIG. 2A

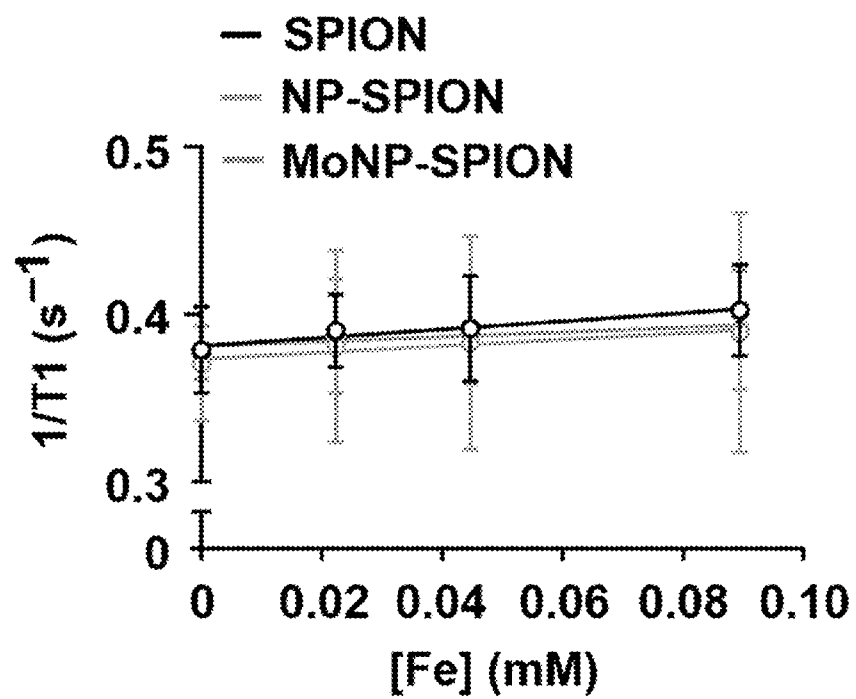


FIG. 2B

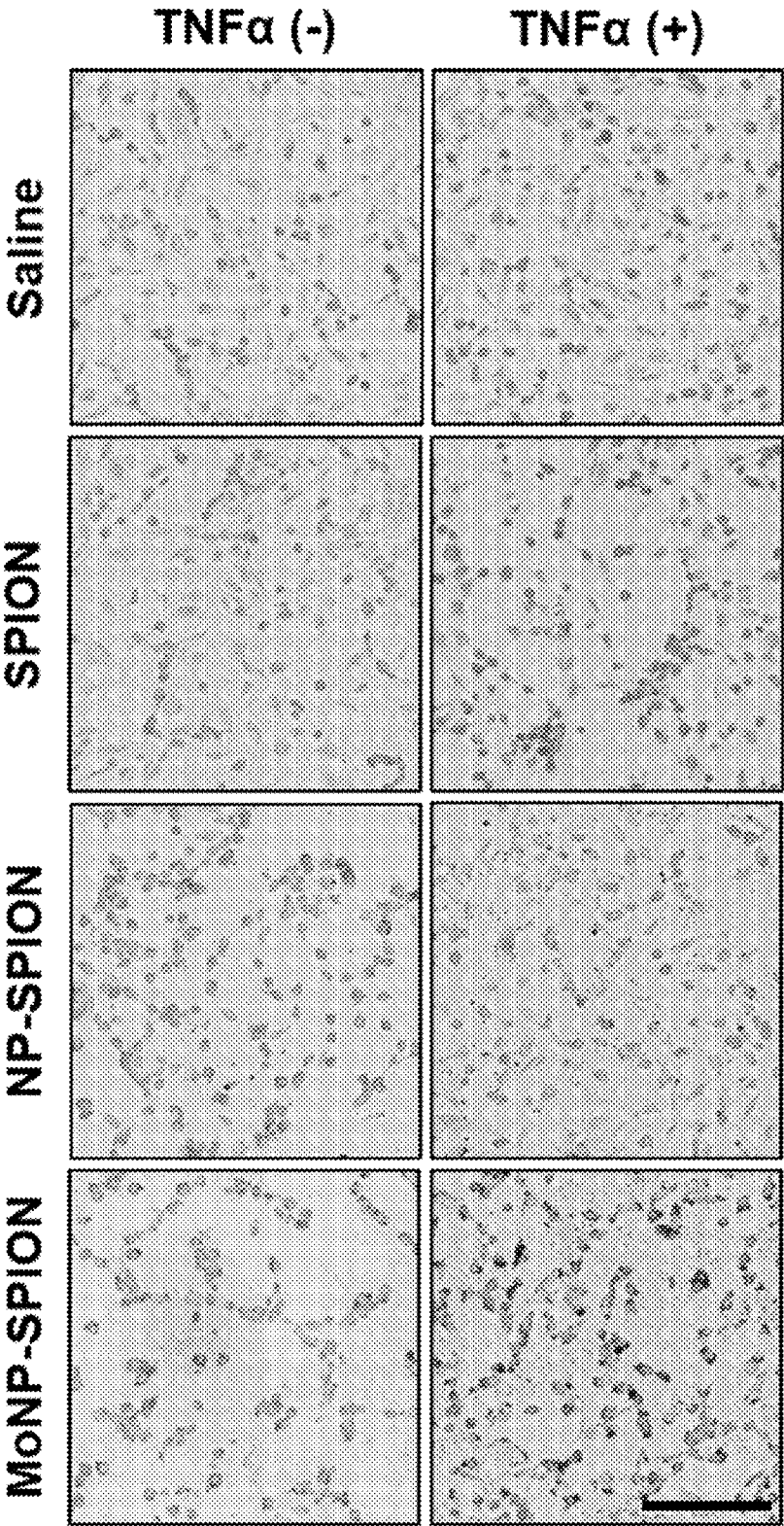


FIG. 3A

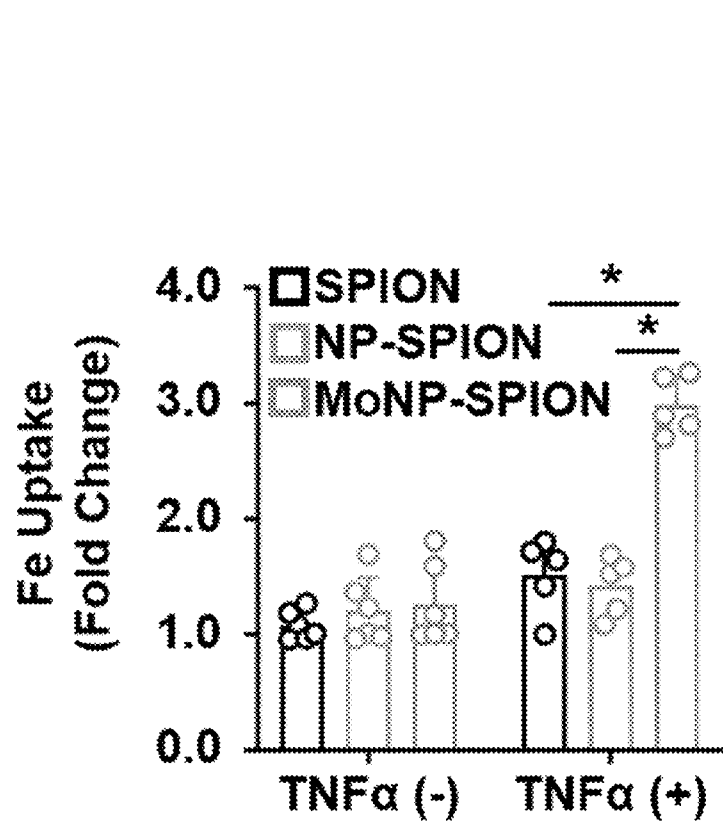


FIG. 3B

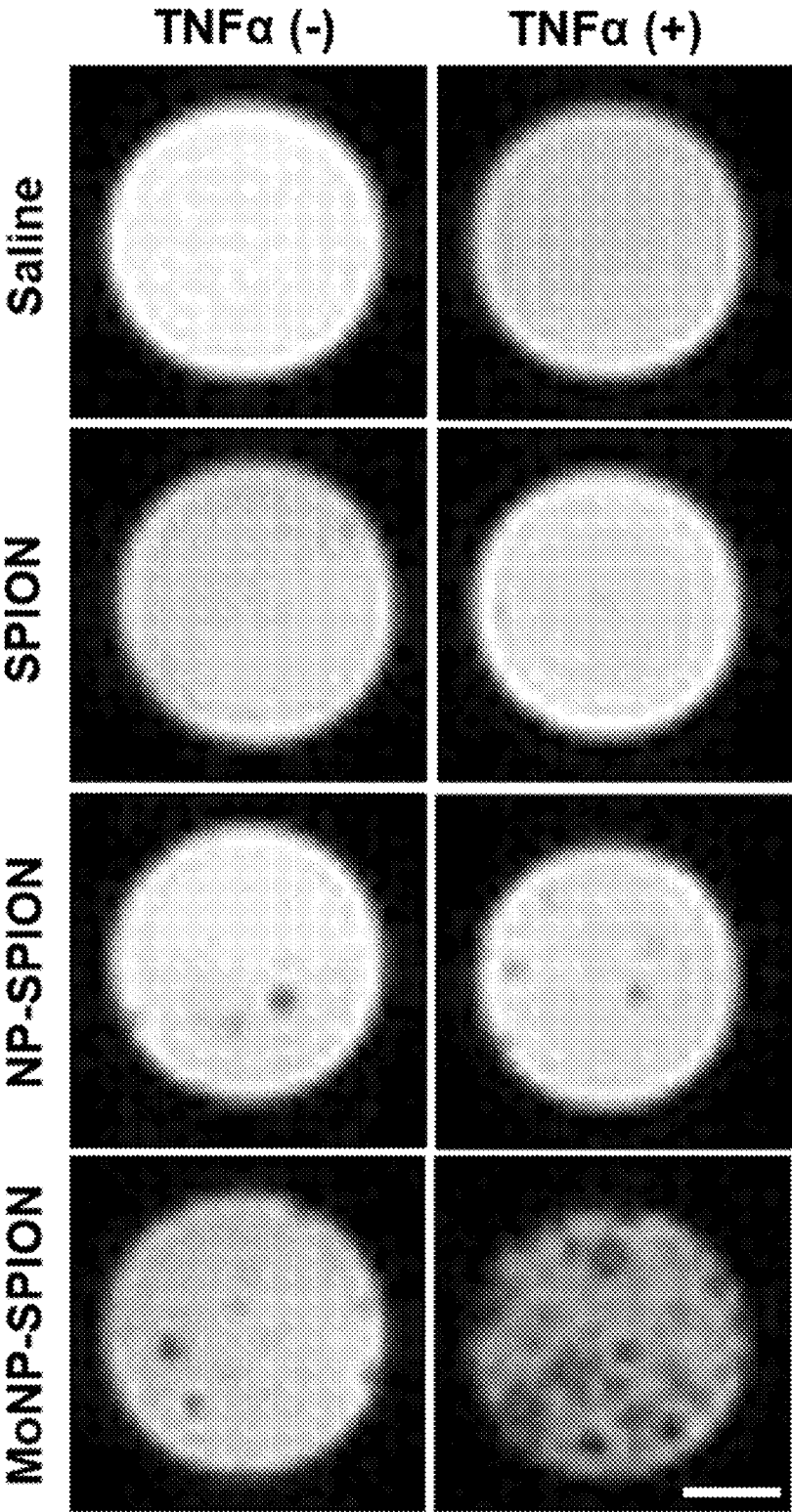


FIG. 3C

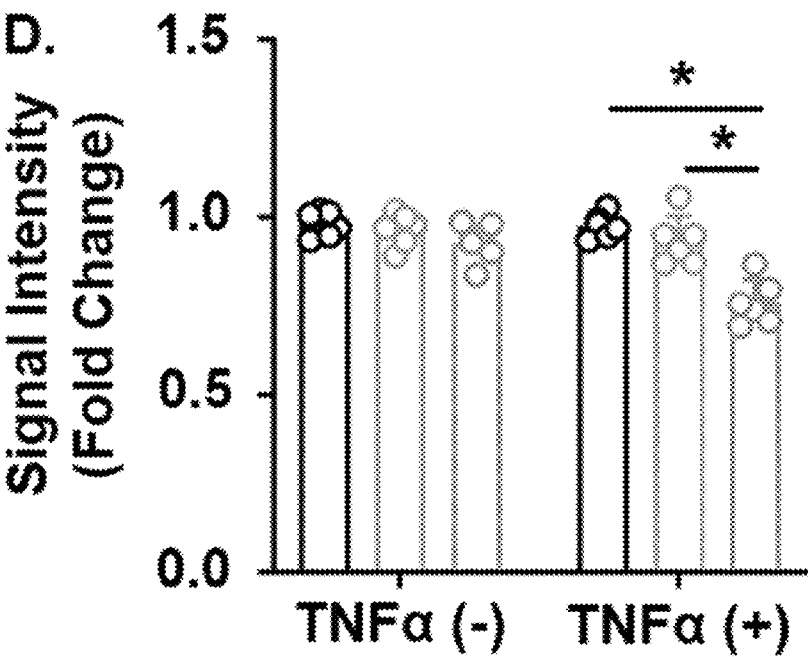


FIG. 3D

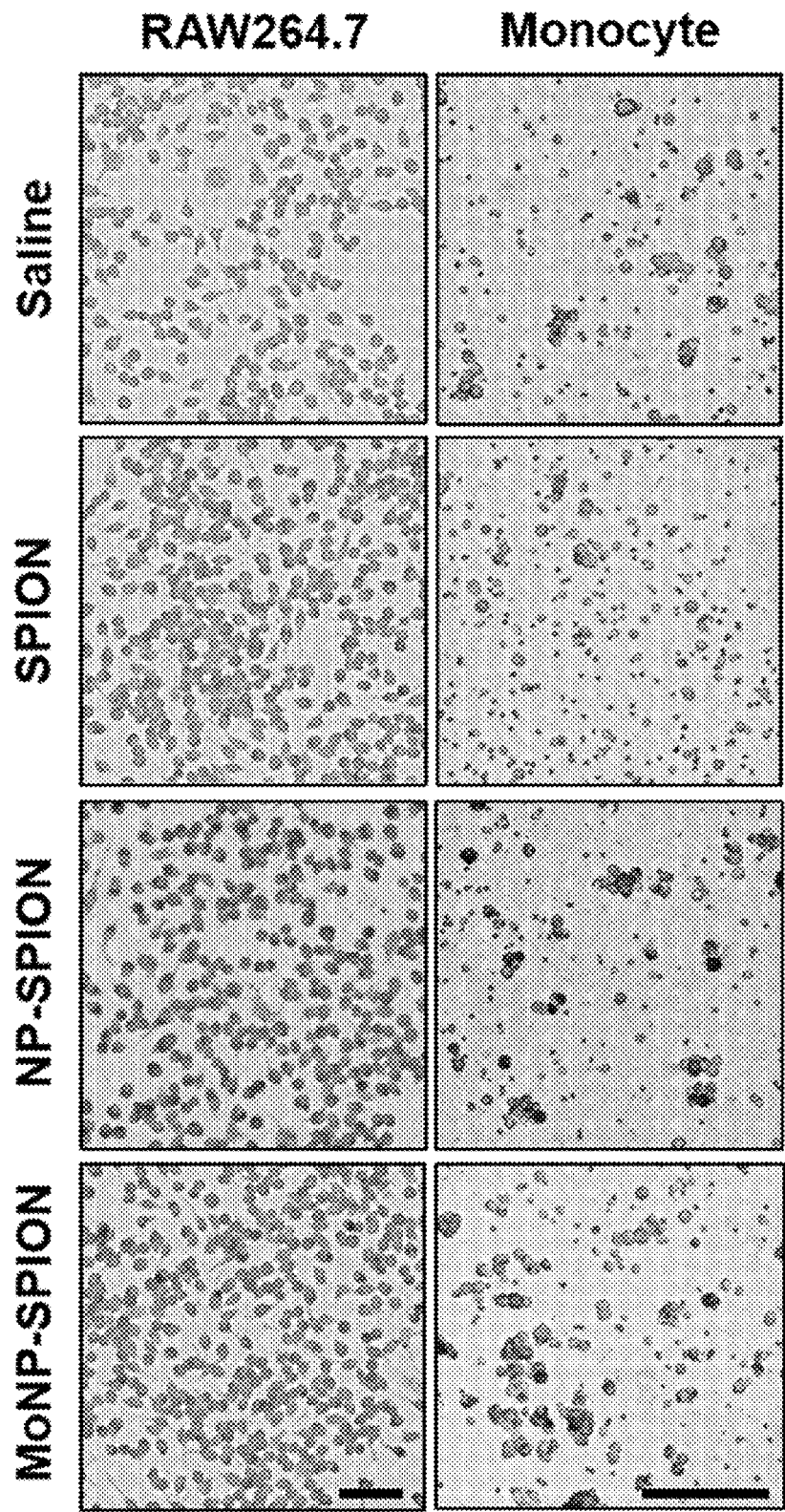


FIG. 3E

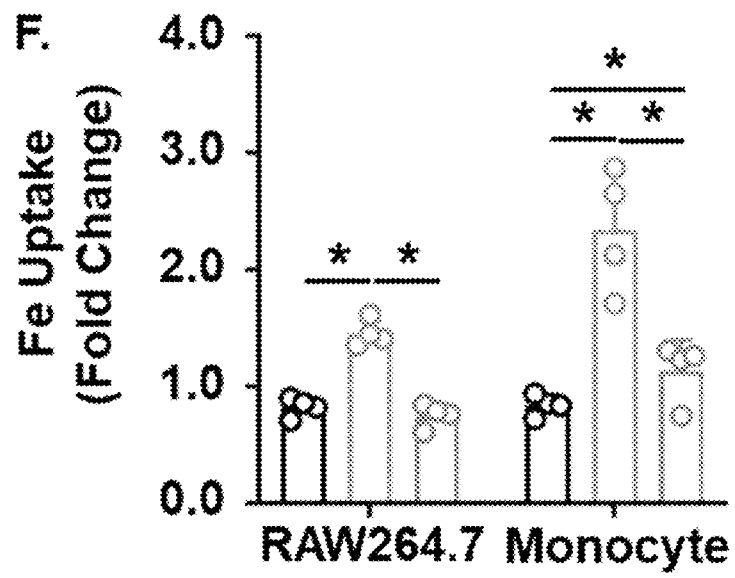


FIG. 3F

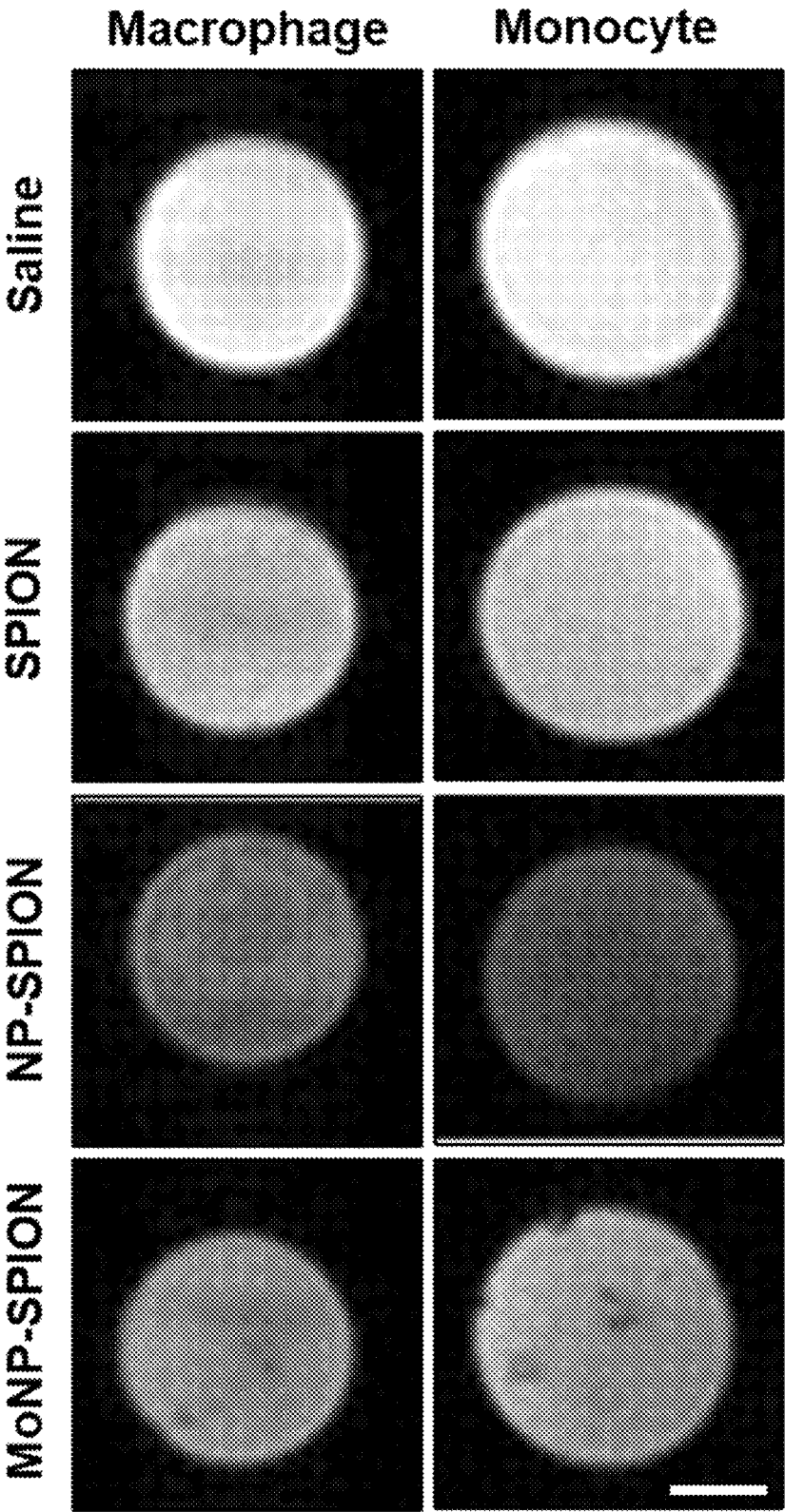


FIG. 3G

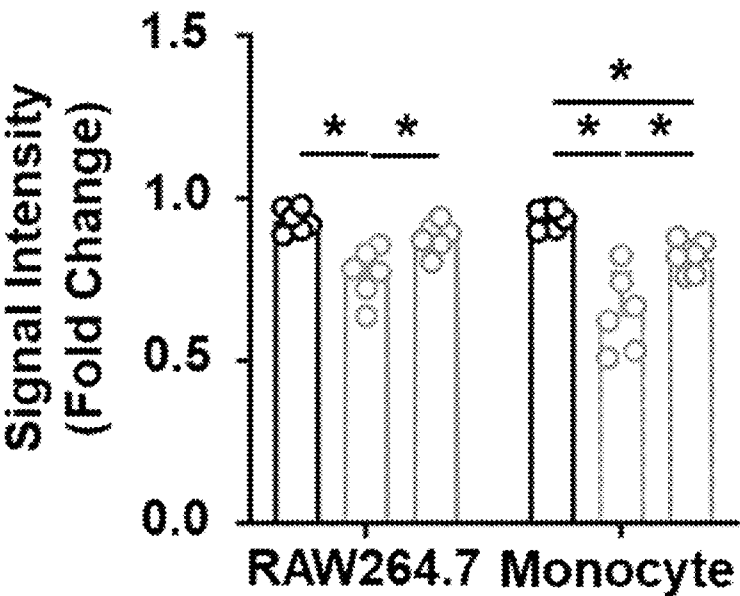


FIG. 3H

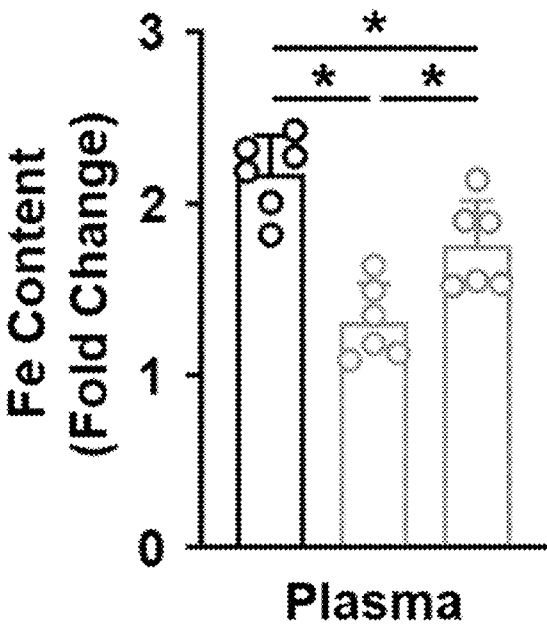


FIG. 3I

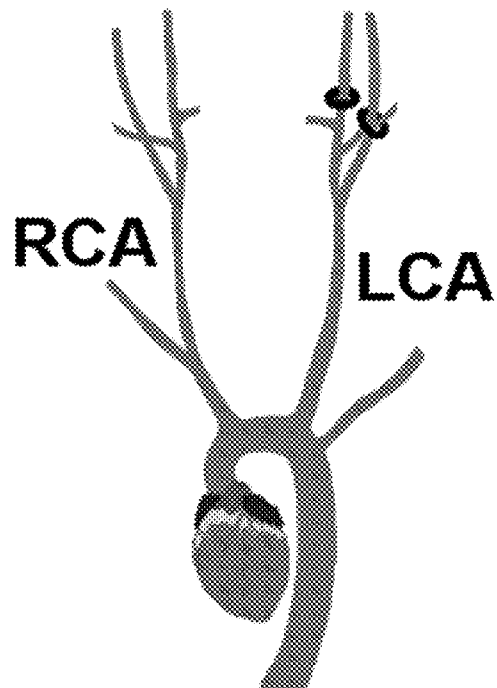


FIG. 4A

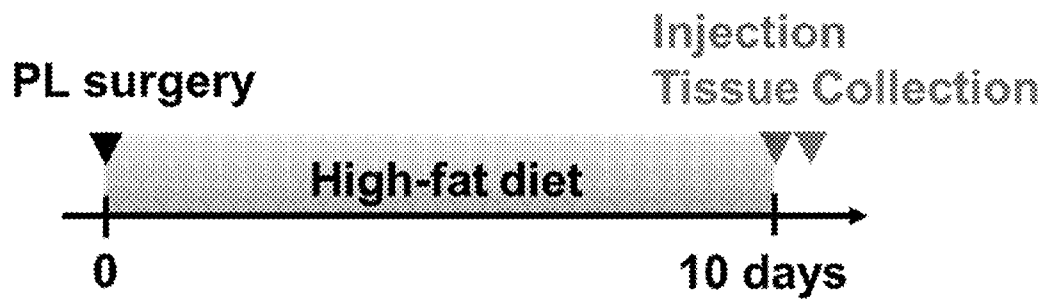


FIG. 4B

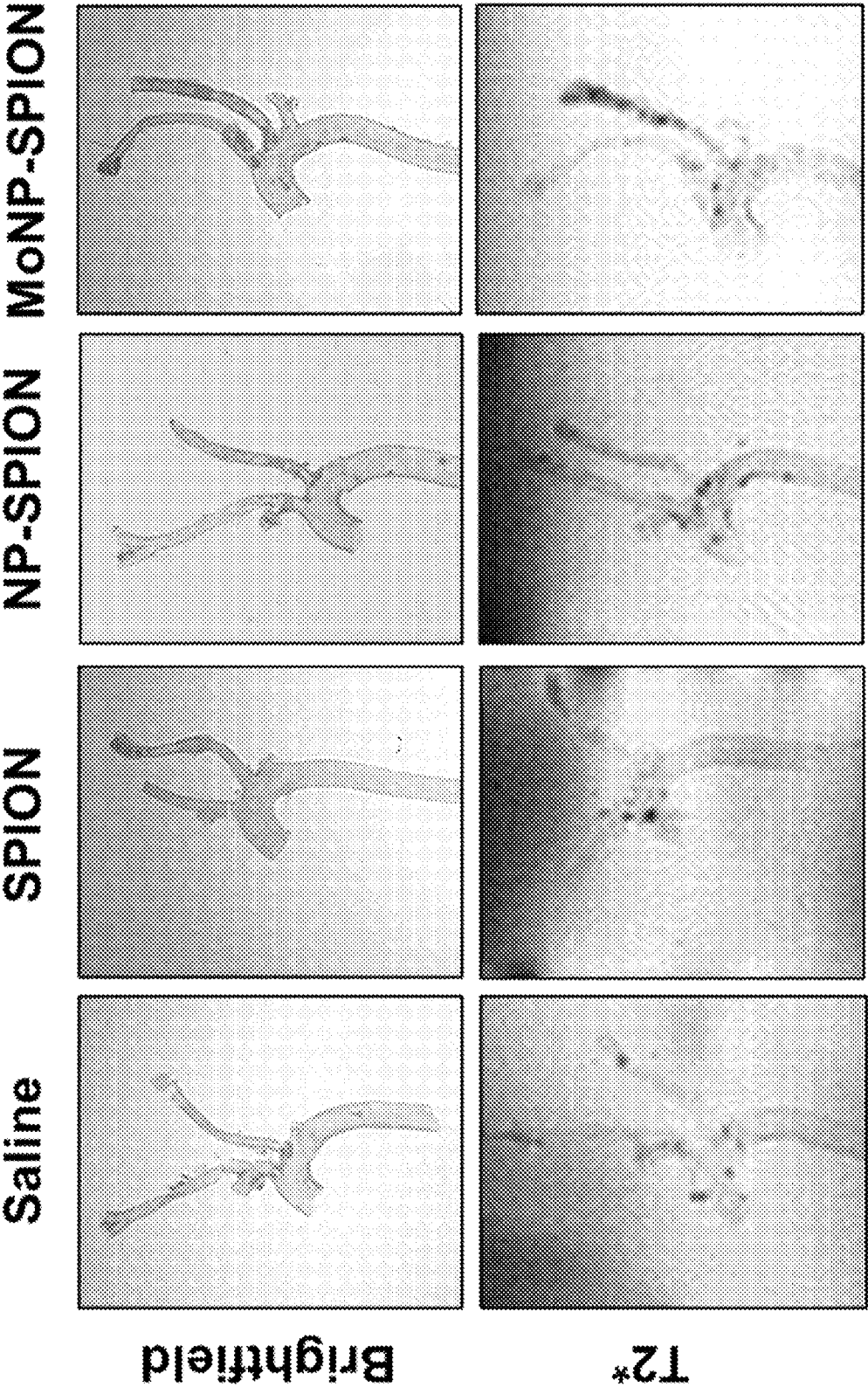


FIG. 4C

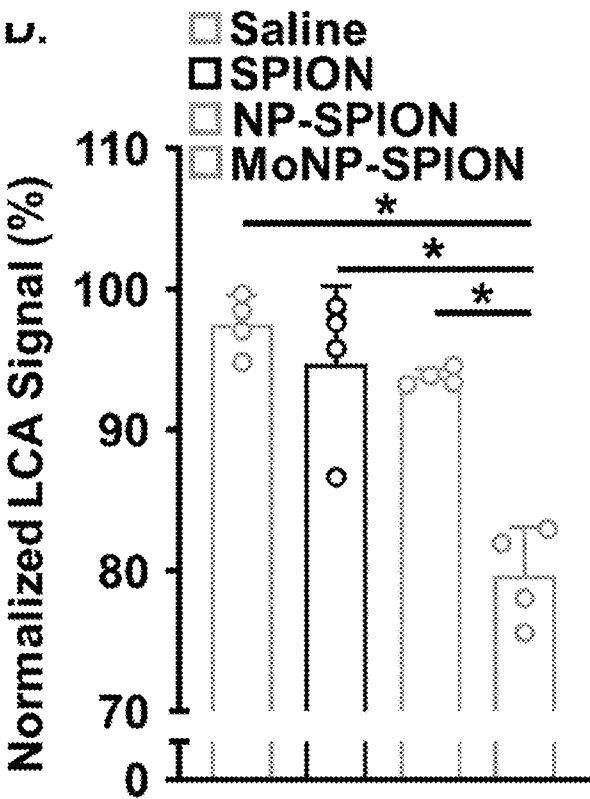


FIG. 4D

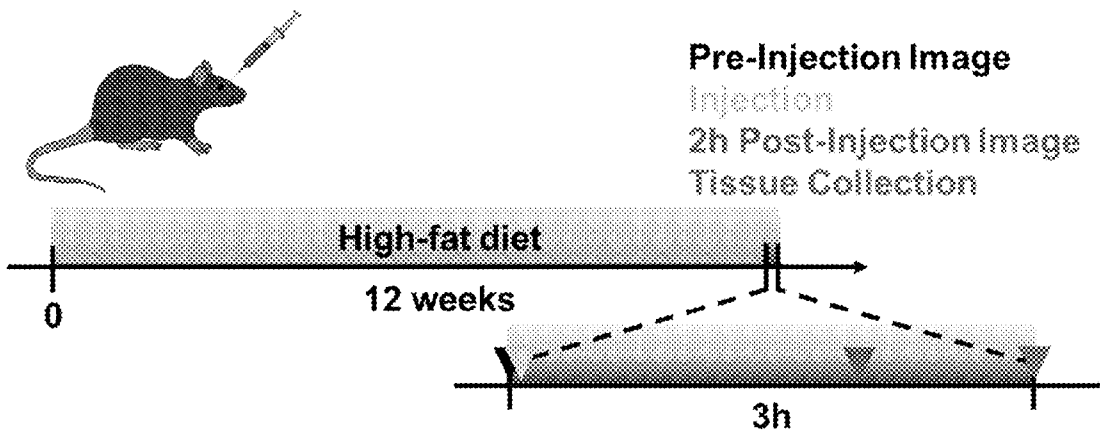


FIG. 5A

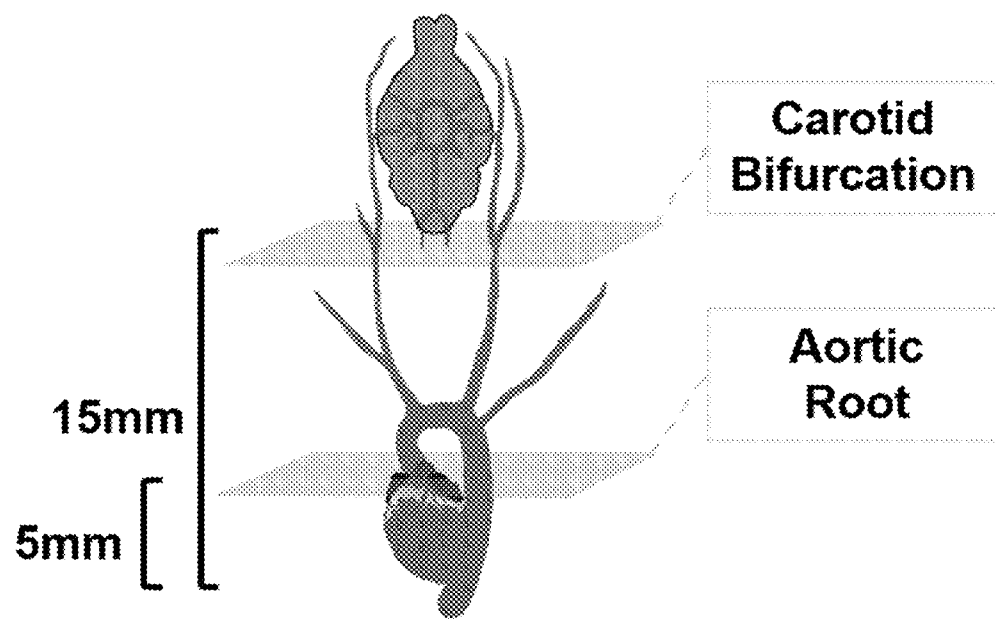


FIG. 5B

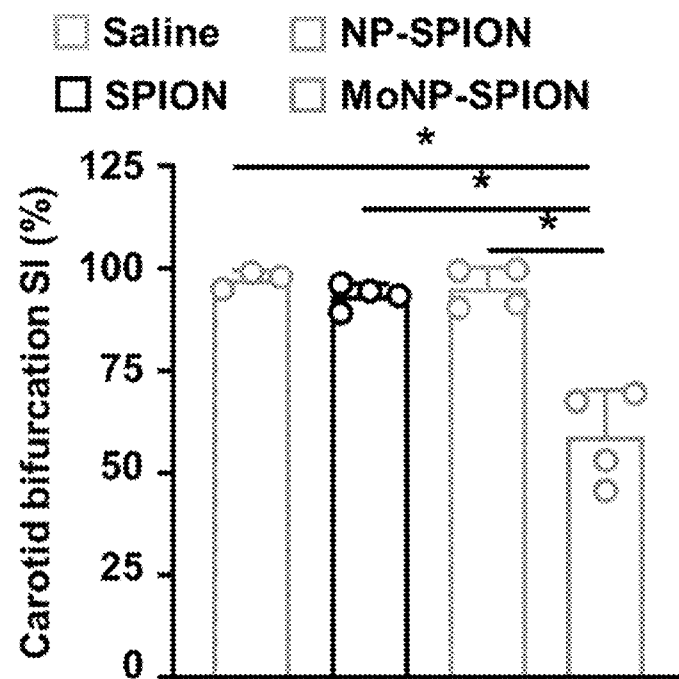
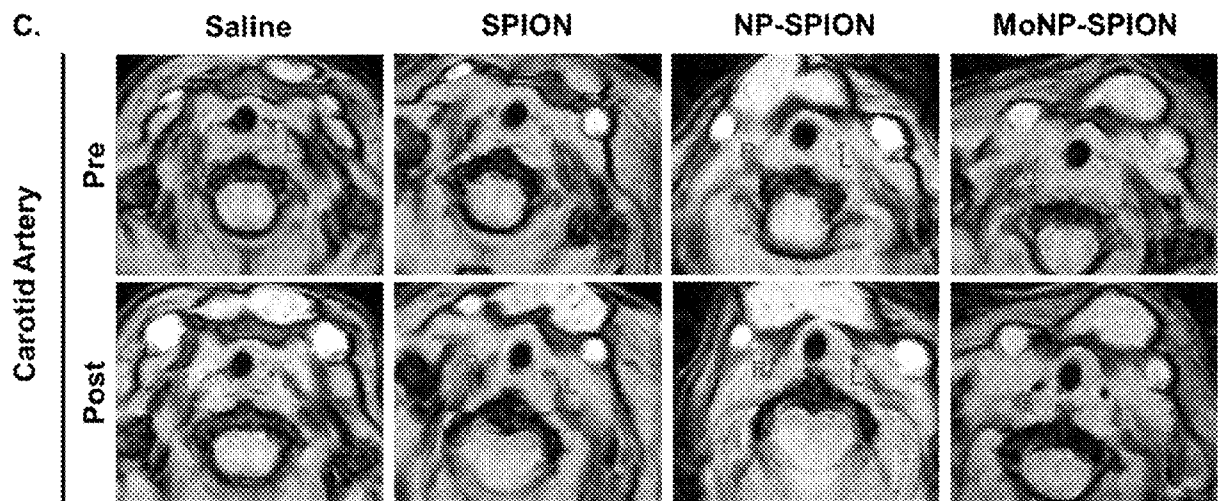


FIG. 5C

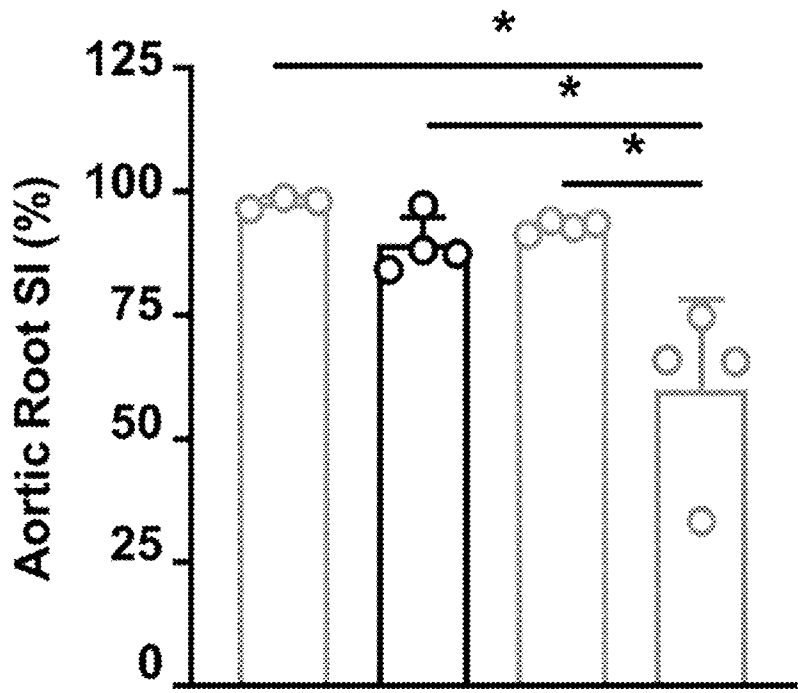
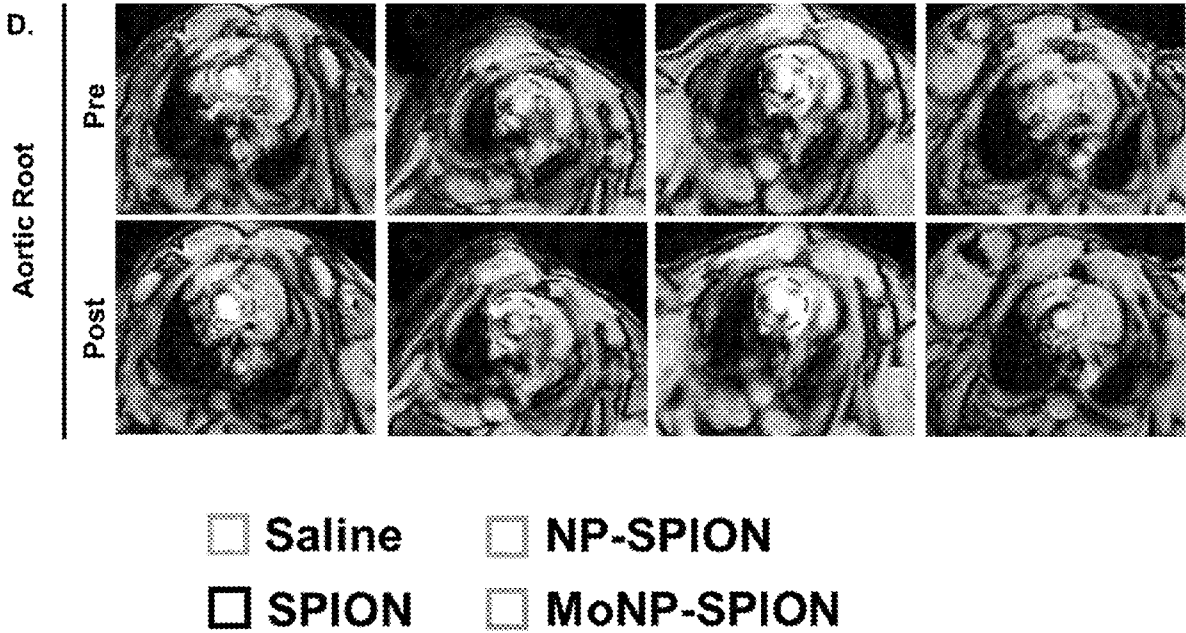


FIG. 5D

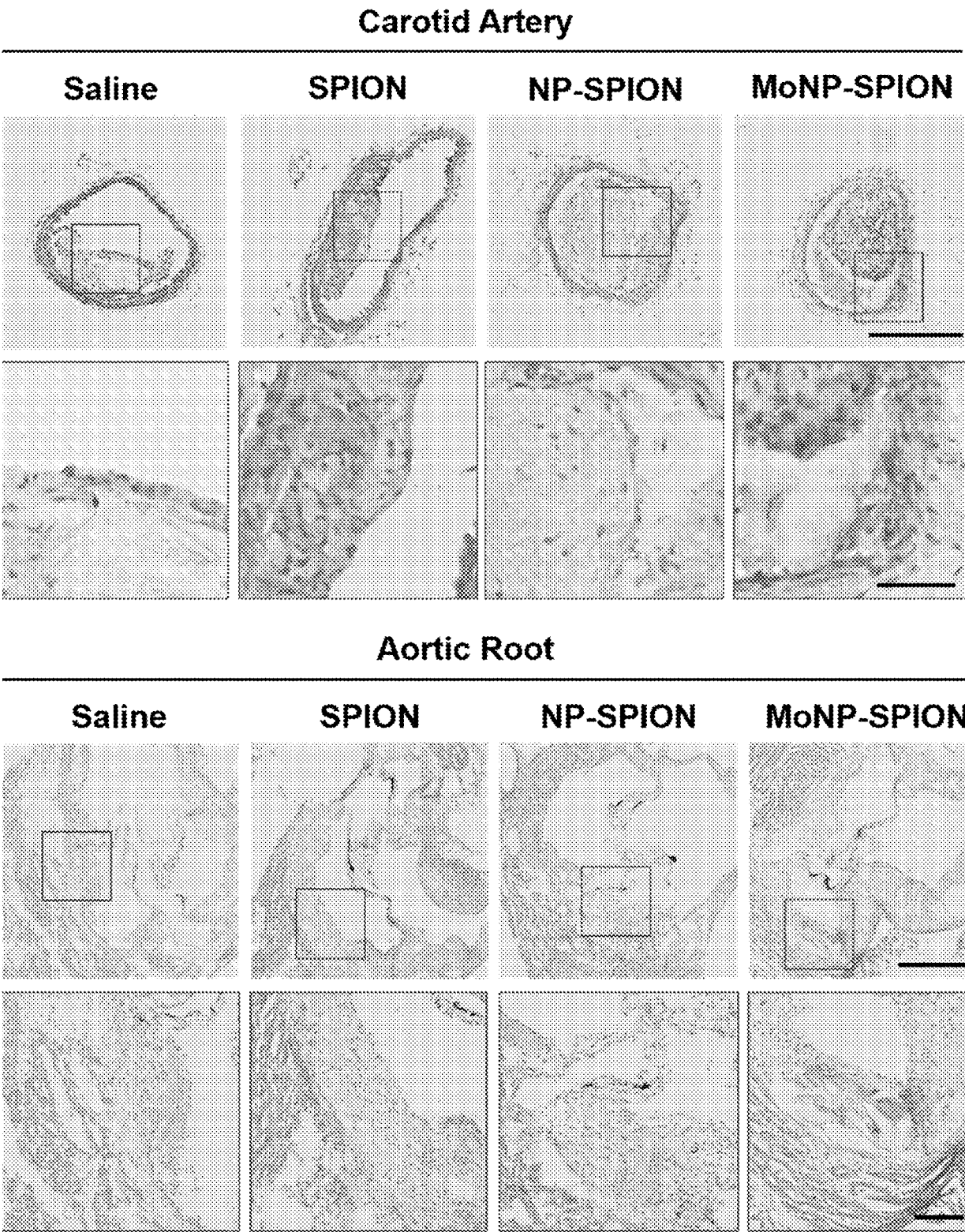


FIG. 5E

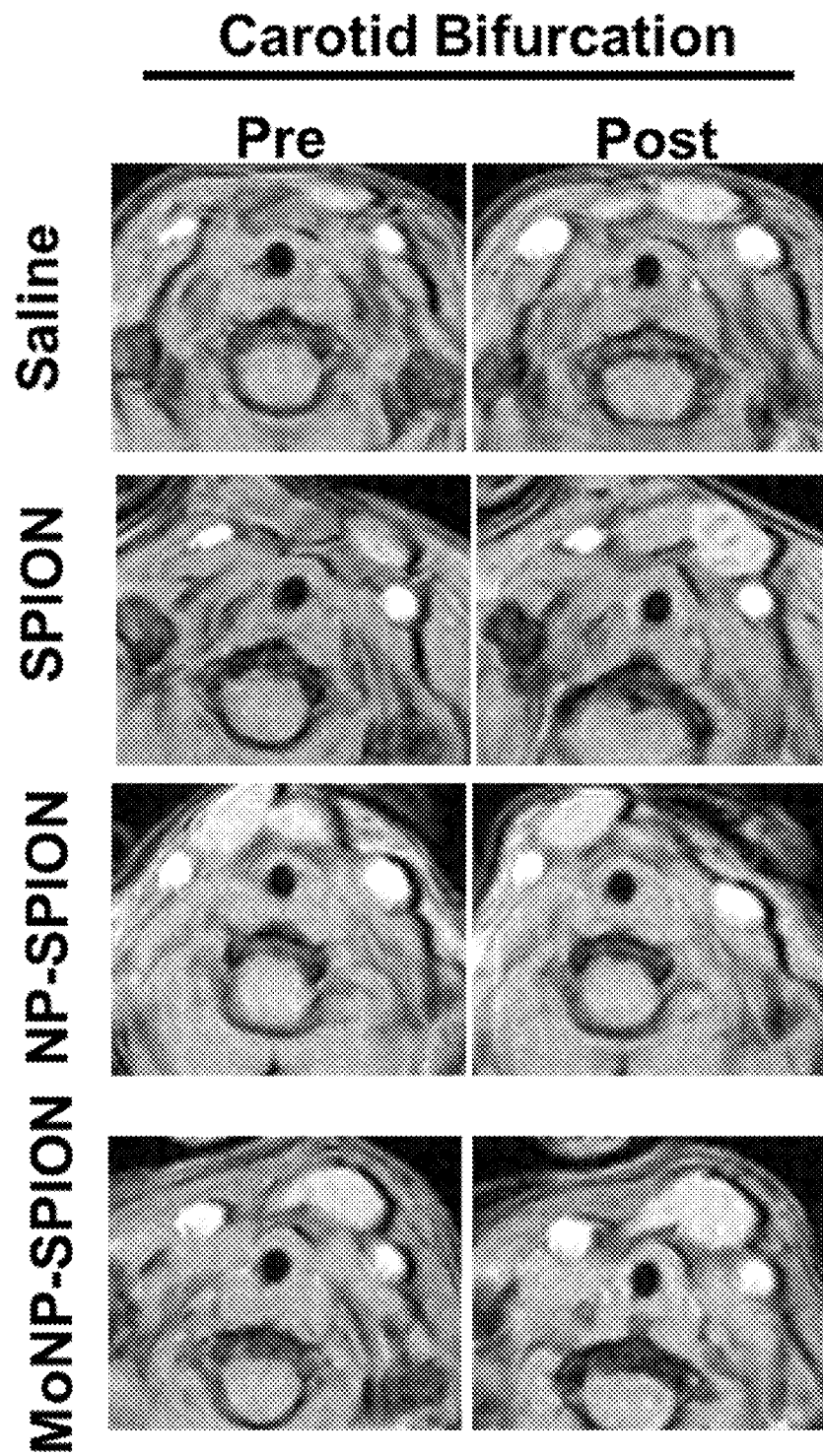


FIG. 6A

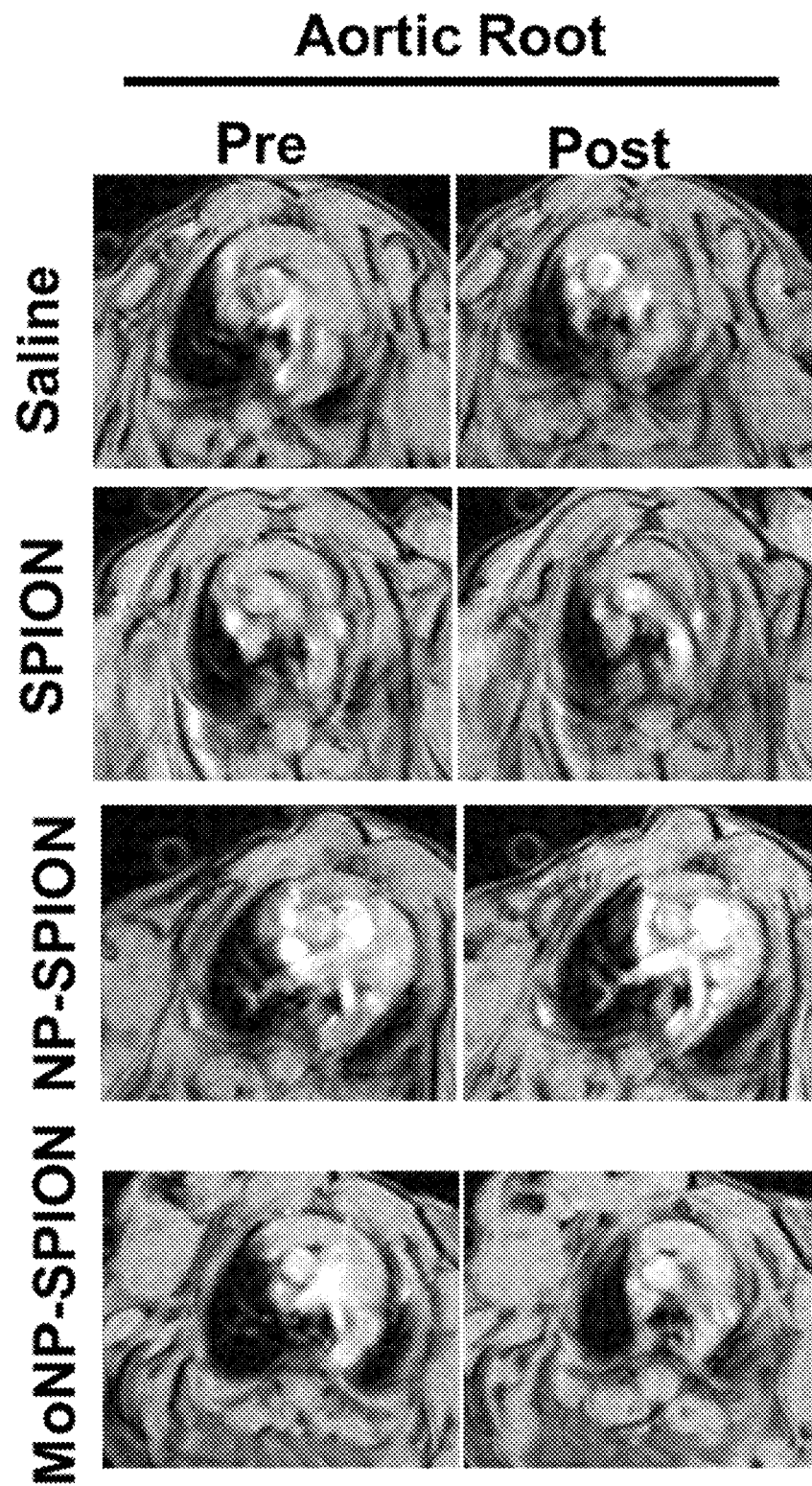


FIG. 6B

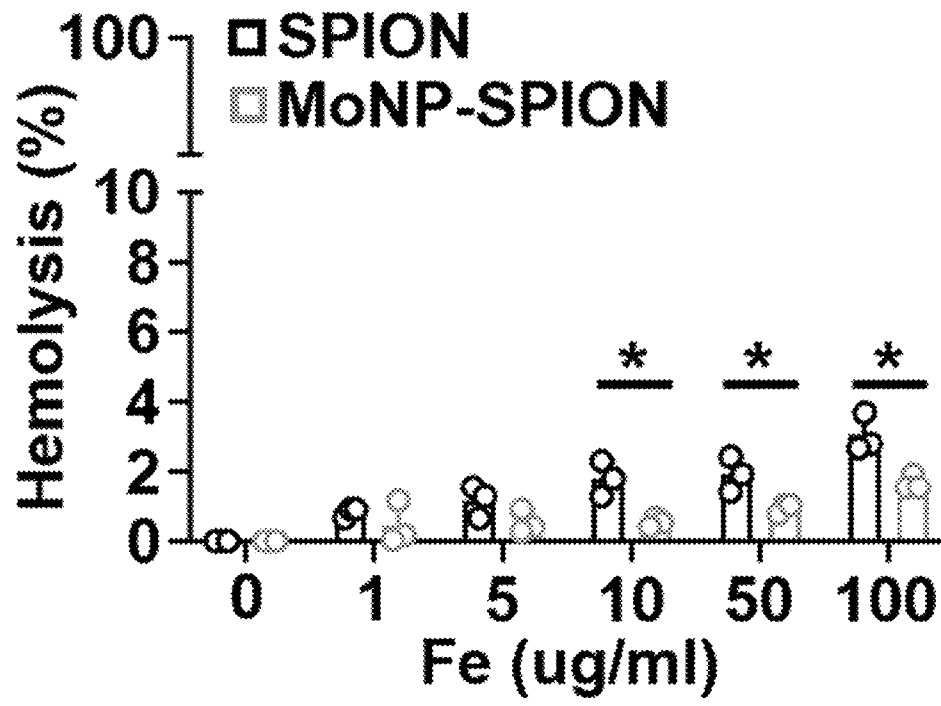


FIG. 7A

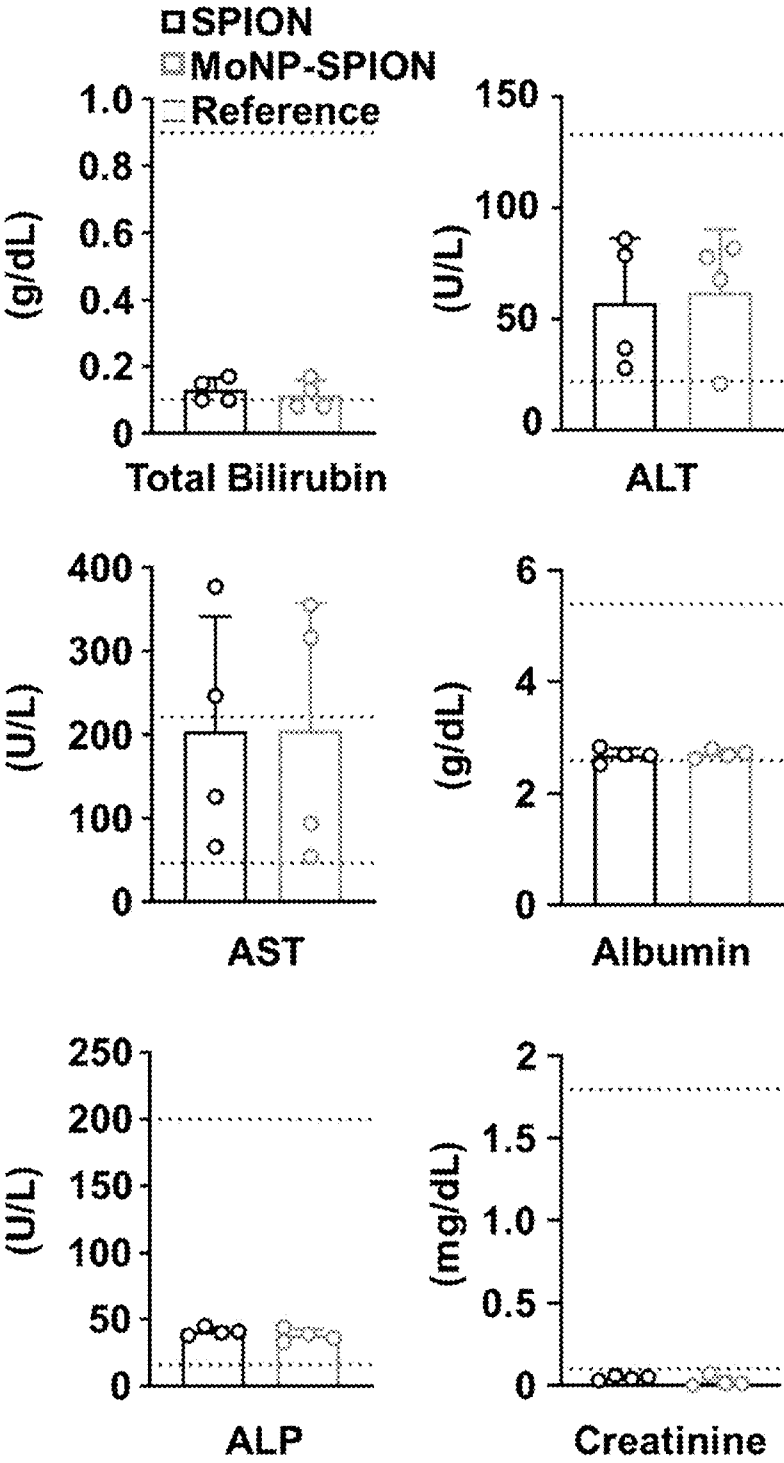


FIG. 7B

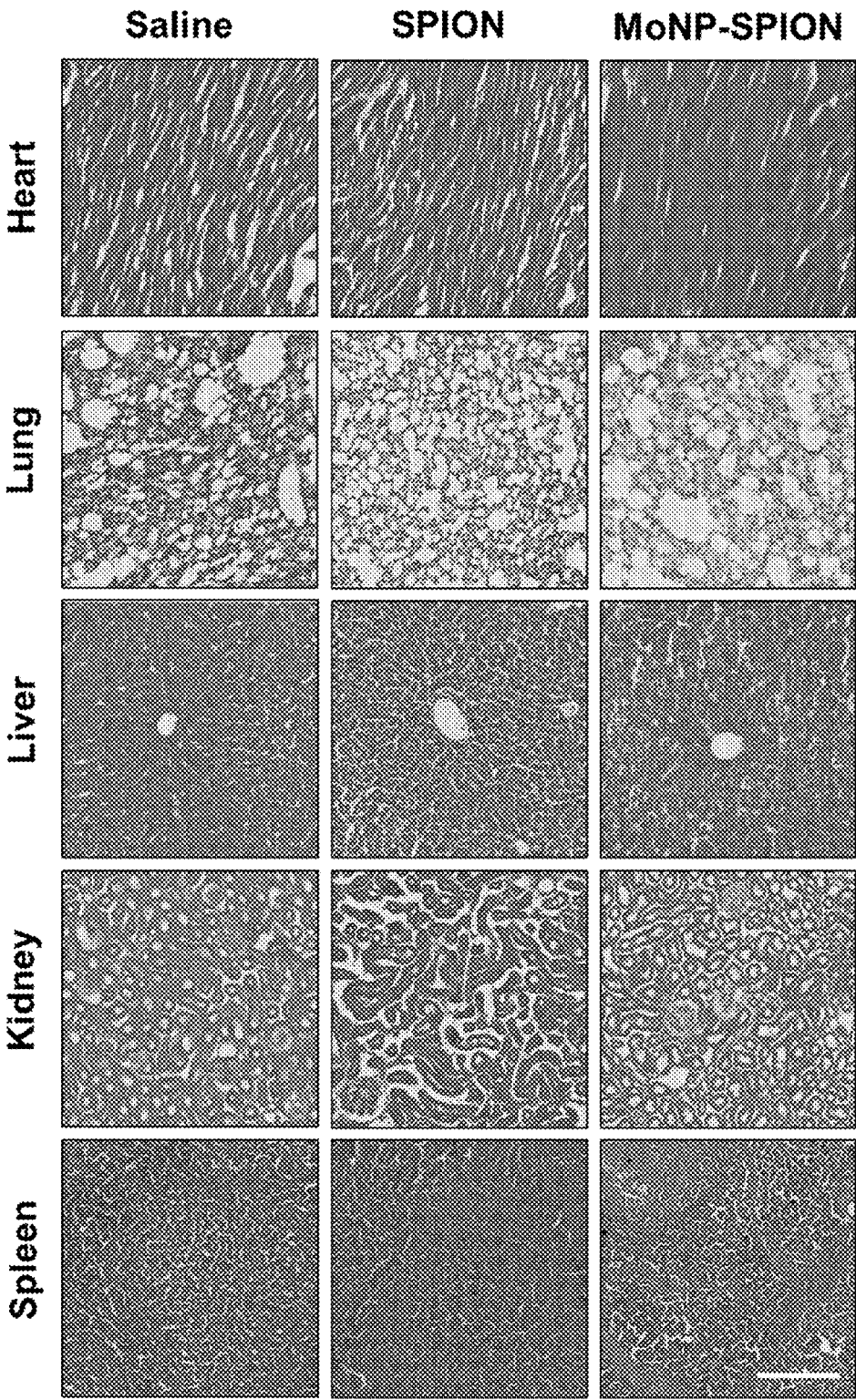


FIG. 7C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/051807

A. CLASSIFICATION OF SUBJECT MATTERIPC: **A61K 49/18** (2023.01); **A61K 49/00** (2023.01); **B82Y 5/00** (2023.01)CPC: **A61K49/1839**; **A61K49/0002**; **A61K49/1812**; **A61K49/1821**; **A61K49/1896**; **B82Y5/00**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2023/049493 A1 (OREGON STATE UNIVERSITY) 30 March 2023 (30.03.2023) Page 120	10
X Y	JAN. "Biomimetic cell membrane-coated poly(lactic-co-glycolic acid) nanoparticles for biomedical applications" 1-36. Bioengineering and Traditional Medicine. Web. 02 February 2022; DOI: 10.1002/btm2.10441 Page 4, 6, 8-9, 14, 22 and 23; Fig 8, 9	1, 3-9, 11, 16-20 2, 10, 12-15
Y	KRISHNAMURTHY. "Monocyte cell membrane-derived nanoghosts for targeted cancer therapy" 6981-6985. Nanoscale. Web. 03 March 2016; DOI: 10.1039/c5nr07588b Page 6982	2, 12-15

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

* Special categories of cited documents:

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Date of the actual completion of the international search

19 November 2024 (19.11.2024)

Date of mailing of the international search report

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