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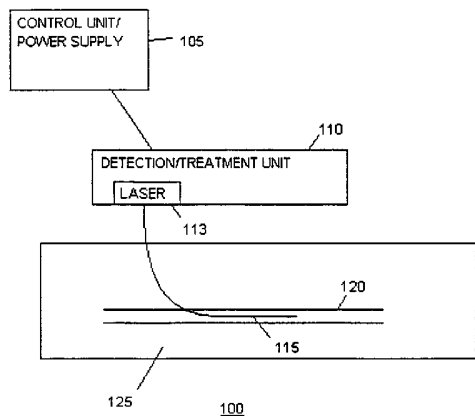
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- (71) Applicant (for all designated States except US): **THE GENERAL HOSPITAL CORPORATION** [US/US]; 55 Fruit Street, Boston, MA 02114 (US).

- (72) Inventors; and
(75) Inventors/Applicants (for US only): **HAMBLIN,**

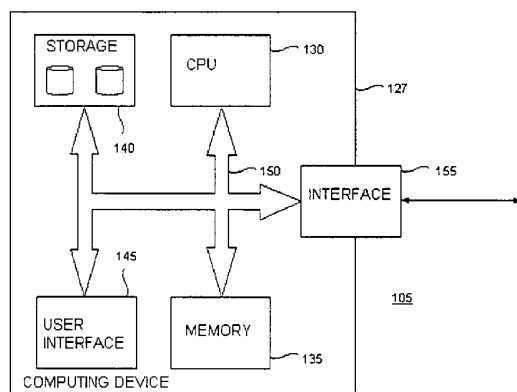
Michael, R. [US/US]; Wellman Laboratories of Photomedicine, Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, 55 Fruit Street, BAR 314B, Boston, MA 02114 (US). **TAWAKOL, Ahmed** [US/US]; C/o Wellman Laboratories of Photomedicine, Department of Cardiology, Harvard Medical School, Massachusetts General Hospital, Nuclear Cardiology, VBK 301, 55 Fruit Street, Boston, MA 02114 (US). **HASSAN, Tayaba** [US/US]; C/o Wellman Laboratories of Photomedicine, Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, 55 Fruit Street, WEL-224, Boston, MA 02114 (US). **FISCHMAN, Alan** [US/US]; C/o Wellman Laboratories of Photomedicine, Department of Radiology, Harvard Medical School, Massachusetts General Hospital, Nuclear Medicine, Tilton-207, 55 Fruit Street, Bartlett 508, Boston, MA 02114 (US). **MULLER, James** [US/US]; C/o Wellman Laboratories of Photomedicine, Department of Cardiology, Harvard Medical School, Massachusetts General Hospital, 55 Fruit Street, GRB-08-840, Boston,

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- (54) Title: DETECTION AND THERAPY OF VULNERABLE PLAQUE WITH PHOTODYNAMIC COMPOUNDS



(57) Abstract: The present invention relates to methods for selectively targeting Photodynamic Therapy ("PDT") to inflammatory components of vulnerable plaques. As such, the present invention provides methods for the identification of vulnerable plaques, as well as methods to treat vulnerable plaques by selectively targeting and/or eliminating the inflammatory components of vulnerable plaques. The invention further relates to a specially designed intravascular device that delivers excitation light to the plaque surface inside the artery and receives emitted fluorescence light when a fluorescent signal is detected.



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MA 02114 (US). **ANDERSON, Rox** [US/US]; Wellman Laboratories of Photomedicine, Dept. of Dermatology, Harvard Medical School, Massachusetts General Hospital, Ste 501, Dermatology Laser Center, 275 Cambridge Street, Boston, MA 02114 (US).

(74) **Agent: KOWALSKI, Thomas, J.**; Frommer Lawrence & Haug LLP, 745 Fifth Avenue, New York, NY 10151 (US).

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TITLE OF THE INVENTIONDETECTION AND THERAPY OF VULNERABLE PLAQUE WITH
PHOTODYNAMIC COMPOUNDSRELATED APPLICATIONS/PATENTS & INCORPORATION BY REFERENCE

This application claims priority to U.S. Provisional Application No. 60/295,627, filed June 4, 2001, and U.S. Provisional Application No. 60/365,673, filed March 15, 2002, the contents of which are expressly incorporated herein by reference.

Each of the applications and patents cited in this text, as well as each document or reference cited in each of the applications and patents (including during the prosecution of each issued patent; "application cited documents"), and each of the PCT and foreign applications or patents corresponding to and/or claiming priority from any of these applications and patents, and each of the documents cited or referenced in each of the application cited documents, are hereby expressly incorporated herein by reference. More generally, documents or references are cited in this text, either in a Reference List before the claims, or in the text itself; and, each of these documents or references ("herein-cited references"), as well as each document or reference cited in each of the herein-cited references (including any manufacturer's specifications, instructions, etc.), is hereby expressly incorporated herein by reference.

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FIELD OF THE INVENTION

The present invention relates to methods for detection and therapy of thin-capped fibro-atheroma ("vulnerable plaque") using selectively targeted photodynamic compounds. The present invention further relates to devices for use in the detection and therapy of vulnerable plaque. Other aspects of the invention are described in or are obvious from the following disclosure (and within the ambit of the invention).

BACKGROUND OF THE INVENTION

Cardiovascular disease remains the leading cause of morbidity and mortality in the United States. A chief contributor to the pathology of the disease is the formation of atherosclerotic or “atheromatous” plaques in the coronary arteries (Farb et al. (1995) *Circulation* 92:1701-1709). Yet, therapies designed to ameliorate the occlusive effects of atheromatous plaques on coronary blood flow, such as coronary artery bypass surgery and percutaneous transluminal coronary angioplasty, do not prevent or reduce the incidence of acute coronary syndrome. Acute coronary syndrome covers a group of sudden-onset coronary diseases, including unstable angina, acute myocardial infarction and sudden cardiac death. The causative agent of acute coronary syndrome is fissure, erosion or rupture of a specific kind of atheromatous plaque known as a “vulnerable plaque.” Vulnerable plaques are responsible for the majority of heart attacks, strokes, and cases of sudden death.

Post-mortem evidence suggests that vulnerable plaque rupture occurs in areas of the coronary arteries that are less than about 50% stenosed. Thus, angioplasty and bypass procedures, which are carried out on severely stenosed arteries, rarely remove vulnerable plaques or reduce the incidence of acute coronary syndrome (Plutzky (1999) *Am J Cardiol* 84:15J-20J). Even with currently available therapeutic approaches, such as lipid lowering, angioplasty and bypass, an unacceptably high incidence of acute coronary syndrome remains (Sacks et al. (2000) *Circulation* 102:1893-1900).

Atheromatous plaques characteristically comprise a fibrous cap surrounding a central core of extracellular lipids and debris located in the central portion of the thickened vessel intima, which is known as the “atheroma.” On the luminal side of the lipid core, the fibrous cap is comprised mainly of connective tissues, typically a dense, fibrous, extracellular matrix made up of collagens, elastins, proteoglycans and other extracellular matrix materials. At the edges of the fibrous cap overlying the lipid core is the shoulder region, enriched with macrophages. The macrophages continually phagocytose oxidized LDL through scavenger receptors, which have a high ligand specificity for oxidized LDL. Continuous phagocytosis results in the formation of foam cells, a hallmark of the atherosclerotic plaque (Parthasarathy et al. (1992) *Annu Rev Med* 43:219-225). Foam cells, together with the binding of extracellular lipids to

collagen fibers and proteoglycans, play an important role in the formation and growth of the lipid-rich atheroma.

Histopathologic examination of atheromatous plaques has revealed substantial variations in the thickness of fibrous caps, the size of the atheromas, the extent of dystrophic calcification and the relative contribution of major cell types (van der Wal et al. (1994) *Coron Artery Dis* 5:463-469). Resident cells present in atheromatous plaques include a significant population of inflammatory cells, such as monocytes/macrophages and T lymphocytes. The emigration of monocytes into the arterial wall, and their subsequent differentiation into macrophages and ultimately foam cells, remains one of the earliest steps in plaque formation. Once there, these cells play a critical role in secreting substances that further contribute to atherosclerosis.

A vulnerable plaque is structurally and functionally distinguishable from a stable atheromatous plaque. For example, several histologic features distinguish a vulnerable plaque from a stable atheromatous plaque. A vulnerable plaque is characterized by an abundance of inflammatory cells (e.g., macrophages and/or T cells), a large lipid pool, and a thin fibrous cap. An atheromatous plaque refers to a wide range of coronary lesions, from subtle collections of lipid, to obstructive coronary lesions that cause angina.

In contrast to vulnerable plaques, the vast majority of atheromatous plaques lay silent. Only the rare atheromatous lesion causes heart attacks or strokes. Pathologic studies have provided a further understanding of why vulnerable plaques have a higher propensity for rupture than other atheromatous plaques. The thickness and integrity of the fibrous cap overlying the lipid-rich core is a principal factor in the stability of the plaque. Vulnerable plaques prone to rupture can be characterized as having thinner fibrous areas, increased numbers of inflammatory cells (e.g., macrophages and T cells), and a relative paucity of vascular smooth muscle cells. Vascular smooth muscle cells are the major source of extra cellular matrix production, and therefore, the absence of vascular smooth muscle cells from a vulnerable plaque contributes to the lack of density in its fibrous cap.

While the fibrous tissue within the cap provides structural integrity to the plaque, the interior of the atheroma is soft, weak and highly thrombogenic. It is rich in

extracellular lipids and substantially devoid of living cells, but bordered by a rim of lipid-laden macrophages (van der Wal et al. (1999) *Cardiovasc Res* 41:334-344). The lipid core is a highly thrombogenic composition, rich in tissue factor, which is one of the most potent procoagulants known. The lesional macrophages and foam cells produce a variety of procoagulant substances, including tissue factor. The fibrous cap is the only barrier separating the circulation from the lipid core and its powerful coagulation system designed to generate thrombus. Essentially, the rapid release of procoagulants into the blood stream at the site of rupture forms an occlusive clot, inducing acute coronary syndrome. Thus, the thinner the fibrous cap, the greater the instability of the thrombogenic lipid core and the greater the propensity for rupture and thrombosis.

Several factors can contribute to the weakened state of the fibrous cap. In particular, inhibition of extracellular matrix production or degradation of extracellular matrix components adversely impacts the structural composition of the fibrous cap. Macrophages and T lymphocytes have been identified as the dominant cell types at the site of plaque rupture or superficial erosion, and each of these inflammatory cells contributes to the inhibitory and/or degradative pathways. Accelerated degradation of collagen and other matrix components is carried out by macrophage proteases, such as matrix metalloproteinases ("MMPs"), which are secreted at the site of the plaque. MMPs constitute an extensive family of enzymes, including interstitial collagenase (e.g., MMP-1), gelatinases (e.g., MMP-2, MMP-9), and stromelysin (e.g., MMP-3). Stromelysins can activate other members of the MMP family, causing degradation among many matrix constituents. The presence of T cells in the plaque can further contribute to weakening of the fibrous cap. Activated T cells produce and secrete interferon- γ , a potent inhibitor of collagen synthesis. Thus, the T lymphocytes represent a potentially large source of interferon- γ that can negatively regulate matrix production. Plaque rupture sites are further characterized by expression of major histocompatibility complex genes, (e.g., human lymphocyte antigen-DR on inflammatory cells and adjacent smooth muscle cells), indicating an active inflammatory reaction that also weakens the fibrous cap.

Present methods of plaque detection, several of which are discussed herein, are inadequate for the identification of vulnerable plaques. Common methods of plaque detection include angiography and angioscopy. Except in rare circumstances angiography gives almost no information about characteristics of plaque components. Angiography is only sensitive enough to detect hemodynamically significant lesions (>70% stenosis), which account for approximately 33% of acute coronary syndrome cases. Angioscopy is a technique based on fiber-optic transmission of visible light that provides a small field of view with relatively low resolution for visualization of interior surfaces of plaque and thrombus. Because angioscopic visualization is limited to the surface of the plaque, it is insufficient for use in detecting vulnerable plaques.

Several methods are being investigated for their ability to identify vulnerable plaques. However, none has proven to be sufficiently sensitive. One such method, intravascular ultrasound ("IVUS") uses miniaturized crystals incorporated at catheter tips and provides real-time, cross-sectional and longitudinal, high-resolution images of the arterial wall with three-dimensional reconstruction capabilities. IVUS can detect thin caps and distinguish regions of intermediate density (e.g., intima that is rich in smooth muscle cells and fibrous tissue) from echolucent regions, but current technology does not determine which echolucent regions are composed of cholesterol pools rather than thrombosis, hemorrhage, or some combination thereof. Moreover, the spatial resolution (i.e., approximately 200 μ m) does not distinguish the moderately thinned cap from the high risk cap (i.e., approximately 25-75 μ m) and large dense calcium deposits produce acoustic echoes which "shadow" so that deeper plaque is not imaged.

Intravascular thermography is based on the premise that plaques with dense macrophage infiltration give off more heat than non-inflamed plaque (Casscells et al. (1996) *Lancet*. 347:1447-1451). The temperature of the plaque is inversely correlated to cap thickness. However, thermography may not provide information about eroded but non-inflamed vulnerable lesions.

Optical coherence tomography ("OCT") measures the intensity of reflected near-infrared light from tissue. It provides images with high resolution that is approximately 10 to 20 times higher than that of IVUS resolution. OCT is primarily used for assessment of atherosclerotic plaque morphology. However, long image acquisition

time, high costs, limited penetration and a lack of physiologic data render this approach undesirable for detection of vulnerable plaques.

Raman spectroscopy utilizes Raman effect: a basic principle in photonic spectroscopy named after its inventor. Raman effect arises when an incident light excites molecules in a sample, which subsequently scatter the light. While most of this scattered light is at the same wavelength as the incident light, some is scattered at a different wavelength. This shift in the wavelength of the scattered light is called Raman shift. The amount of the wavelength shift and intensity depends on the size, shape, and strength of the molecule. Each molecule has its own distinct "fingerprint" Raman shift. Raman spectroscopy is a very sensitive technique and is capable of reporting an accurate measurement of chemical compounds. Conceivably, the ratio of lipid to proteins, such as collagen and elastin, might help detect vulnerable plaques with large lipid pools. However, it is unlikely that vulnerable plaques will be reliably differentiated from stable plaques based solely on this ratio.

Photodynamic therapy ("PDT") employs photoactivatable compounds known as photosensitizers to selectively target and destroy cells. Therapy involves delivering visible light of the appropriate wavelength to excite the photosensitizer molecule to the excited singlet state. This excited state can then undergo intersystem crossing to the slightly lower energy triplet state, which can then react further by one or both of two pathways, known as Type I and Type II photoprocesses (Ochsner (1997) *J Photochem Photobiol B* 39:1-18). The Type I pathway involves electron transfer reactions from the photosensitizer triplet to produce radical ions which can then react with oxygen to produce cytotoxic species such as superoxide, hydroxyl and lipid derived radicals. The Type II pathway involves energy transfer from the photosensitizer triplet to ground state molecular oxygen (triplet) to produce the excited state singlet oxygen, which can then oxidize many biological molecules such as proteins, nucleic acids and lipids, and lead to cytotoxicity.

Photodynamic therapy (PDT) has recently gained regulatory approval in the United States for treatment of esophageal cancer and in other countries for several other types of cancers (Dougherty et al. (1998) *J Natl Cancer Inst* 90:889-905). Certain photosensitizers accumulate preferentially in malignant tissues (Hamblin & Newman

(1994) J Photochem Photobiol B 23:3-8), creating the advantage of dual selectivity: not only is the photosensitizer ideally specific for the target tissue, but the light can also be accurately delivered to the target tissue, thereby limiting the area within which the toxic effects of the photosensitizer are released.

Photodynamic therapy has been applied in cardiovascular medicine for two broad indications: treatment of atherosclerosis ("photoangioplasty") and inhibition of restenosis due to intimal hyperplasia after vascular interventions (Rockson et al. (2000) Circulation 102:591-596, U.S. Patent Nos. 5,116,864, 5,298,018, 5,308,861, 5,422,362, 5,834,503 and 6,054,449). Hematoporphyrin derivative ("HpD") was the first of a number of photosensitizers with demonstrable, selective accumulation within atheromatous plaques (Litvack et al. (1985) Am J Cardiol 56:667-671). Subsequent studies have underscored the affinity of porphyrin derivatives for atheromatous plaques in rabbits and miniswine. There is maximal photosensitizer accumulation within the arterial intimal surface layers, which is diminished in comparison to the arterial media. Both HpD and Photofrin, a more purified derivative of HpD, also display *in vitro* preferential uptake by human atheromatous plaques. However, there is generally a relative lack of selectivity of most photosensitizers for atheromatous plaques and more particularly for vulnerable plaques. Moreover, methods known in the art for photodynamic destruction of atherosclerotic plaques generally fail as a result of the inflammatory response that follows PDT.

Recently, interventional strategies leading to vulnerable plaque stabilization have become an active area of research (Rabbani & Topol (1999) Cardiovasc Res 41:402-417). A photodynamic therapy designed to detect, stabilize and reduce or eliminate vulnerable plaques without inducing an inflammatory response would be highly desirable.

OBJECT AND SUMMARY OF THE INVENTION

The present invention provides methods for selectively targeting PDT to inflammatory components of vulnerable plaques, such as inflammatory cells, proteases and lipids. As such, the present invention provides methods for the identification of vulnerable plaques. Photochemical methods of the present invention advantageously

differentiate stable atheromatous lesions from vulnerable plaques. Furthermore, the present invention provides methods to treat vulnerable plaques by selectively targeting and eliminating the inflammatory components of vulnerable plaques. Once a vulnerable plaque is identified by methods of the present invention, further methods can be employed to stabilize the plaque against rupture while additionally reducing specific populations of cells (e.g., inflammatory cells such as macrophages and T cells) or other components (e.g., lipids and proteases) within or around the plaque, thus reducing the overall size and severity of the plaque.

In one aspect of the invention, photodynamic compositions can be selectively targeted to inflammatory components within and around the vulnerable plaque (e.g., macrophages, T cells, lipids and proteases). In one embodiment, photodynamic compositions are targeted to macrophages to reduce or eliminate secretion of proteases. Reducing or eliminating protease activity greatly enhances the stability of the fibrous cap and, thus, the vulnerable plaque. In yet another embodiment, photodynamic compositions are targeted to T cells to reduce or eliminate secretion of factors that reduce or inhibit extracellular matrix production, such as interferon- γ . A carefully controlled application of PDT is administered to induce apoptotic cell death in the target cells. Advantageously, the parameters of PDT, including light dosimetry and amount of photodynamic compound, can be controlled to induce only apoptosis and not necrosis of the targeted cells. Inducing apoptosis rather than necrosis reduces or eliminates the inflammatory response following PDT and enhances the overall therapeutic effect.

In yet another aspect of the invention, application of PDT to the vulnerable plaque will induce cross-linking of extracellular matrix proteins (e.g., collagen) to further stabilize the fibrous cap against rupture. Advantageously, the parameters of PDT, including the subcellular location of the photodynamic compounds, can be controlled to optimize clustering of the photodynamic compounds on the cell surface. Under these conditions, PDT induces cell surface cross-linking and not cell necrosis, reducing or eliminating the inflammatory response.

In yet another aspect, photoactivation can be carried out using a specially designed intravascular device that delivers excitation light to the plaque surface inside the artery and receives emitted fluorescence that is transmitted to an analysis

instrument. The same device can optionally be used to deliver therapeutic light when a fluorescent signal is detected.

Other aspects of the invention are described in or are obvious from the following disclosure (and within the ambit of the invention).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A illustrates a detection/treatment system for detecting and/or targeting and/or treating vulnerable plaque in accordance with an embodiment of the invention. Figure 1B is a diagram illustrating a configuration of the control unit of Figure 1A.

Figures 2A and 2B are diagrams showing a probe/catheter in accordance with an embodiment with the present invention. Figures 2C and 2D are diagrams showing alternative views of Figures 2A and 2B, respectively. Figure 2E and 2F illustrate a probe/catheter in accordance with an embodiment of the invention.

Figures 3A, 3B and 3C are diagrams showing a probe/catheter in accordance with an embodiment of the invention.

Figures 4A and 4B show a probe/catheter in accordance with an embodiment of the invention.

Figures 5A and 5B are diagrams illustrating a light delivery element and a light deflection element in accordance with respective embodiments of the invention.

Figures 6A, 6B and 6C illustrate a probe/catheter in accordance with an embodiment of the present invention.

Figure 7 shows the scheme for preparing chlorin e6 photosensitizer conjugates.

Figure 8 shows BSA- c_{e6} purified from unreacted c_{e6}-NHS ester using a Sephadex G50 column and acetone precipitation (8A: Thin Layer Chromatography; 8B: SDS-PAGE gel visualized by fluorescence (left) and Coomassie stain (right) before acetone precipitation; 8C: SDS-PAGE gel visualized by fluorescence (left) and Coomassie stain (right) after acetone precipitation)

Figure 9 shows the UV-visible absorption spectra of the purified mal-BSA-c_{e6} conjugates and free c_{e6}.

Figure 10 shows the selective targeting and phototoxicity of maleylated BSA-c_{e6} conjugates.

Figure 11 shows an optical multichannel analyzer used for fluorescence localization within ex vivo aortas.

Figure 12 shows an analysis of aortic sections from rabbits injected with or without conjugates about 24 hours after injection of the conjugate (Row 1: confocal fluorescence, Red=chlorin e6, Green=elastic lamina auto-fluorescence; Row 2: fluorescence emission spectra of intimal surface of aortic segments ex vivo; Row 3: Hematoxylin and eosin staining of formalin fixed paraffin embedded aortic segments; Row 4: Verhoeff's elastic tissue stain). Column 1 shows an atherosclerotic rabbit with no injection of conjugate. Column 2 shows a normal non-atherosclerotic rabbit injected with conjugate. Column 3 shows an atherosclerotic rabbit injected with conjugate.

Figure 13 shows a significant fluorescent signal from the intimal surface (determined by Skin Scan) in all sections from atherosclerotic rabbits compared to the corresponding sections of aorta from normal rabbits injected with conjugate. (Top: 1=thoracic aorta, 2=upper abdominal aorta below diaphragm, 3=mid abdominal aorta, 4=lower abdominal aorta, 5=pelvic aorta just above bifurcation; Middle: Measurement of intimal surface fluorescence made by OMA-LIF system; Bottom: Data from extraction of gross tissue samples).

Figure 14 shows the contrast between a large aortic plaque and an area of the abdominal aorta 5 mm beneath the plaque (14A), between the balloon injured iliac artery and the contralateral normal artery in the same rabbit (14B), and between the plaque-laden aorta of an atherosclerotic rabbit and the same area of the aorta in a normal rabbit (14C).

Figure 15 shows a laparotomy and surgical exposure of the aorta and surrounding tissues (15A) and a histological examination of the arteries (15B: Top- histopathology of PDT treated atherosclerotic aorta; Bottom- histopathology of atherosclerotic aorta that received light but no conjugate).

DETAILED DESCRIPTION

Methods for Detecting and Treating Vulnerable Plaque

In one aspect, the present invention relates to methods for the treatment of vulnerable plaques by selectively targeting and destroying the inflammatory components of vulnerable plaques. In one embodiment, a method of stabilizing a vulnerable plaque in a subject comprises the steps of:

- a) administering a therapeutically effective amount of at least one photosensitizer composition, wherein the photosensitizer composition is localized to a vulnerable plaque; and
- b) light activating the photosensitizer composition to produce a phototoxic species; and
- c) stabilizing the vulnerable plaque against rupture.

A "vulnerable plaque" comprises an abundance of inflammatory cells, a large lipid pool, and a thin fibrous cap. Preferably, a vulnerable plaque comprises a fibrous cap that is less than about 150 microns thick. More preferably, a vulnerable plaque comprises a fibrous cap that is less than about 100 microns thick (e.g., between about 60 and 100 microns thick). Preferably, a vulnerable plaque comprises a macrophage and/or monocyte content that is greater than about 10%. More preferably, a vulnerable plaque comprises a macrophage and/or monocyte content that is greater than about 25%. Preferably, a vulnerable plaque comprises a lipid content that is greater than about 10%. More preferably, a vulnerable plaque comprises a lipid content that is greater than about 25%.

"Inflammatory components" include inflammatory cells, lipids, procoagulants (e.g., tissue factor) and enzymes or other agents that promote inhibition of extracellular matrix production or degradation of extracellular matrix components (e.g., proteases).

"Inflammatory cells" include smooth muscle cells, leukocytes, lymphocytes (B-lymphocytes and T-lymphocytes), monocytes, macrophages, foam cells, mast cells, endothelial cells, platelets, erythrocytes and polymorphonuclear cells (e.g., granulocytes and neutrophils). As used herein, the term, "thrombus" refers to a clot of blood formed within a blood vessel from a ruptured plaque and which remains attached to its place of origin.

As used herein, "photosensitizer" is a chemical compound, or a biological precursor thereof, that produces a phototoxic or other biological effect on biomolecules

upon photoactivation. A "phototoxic species" is an amount or variety of reactive species that is sufficient to product a phototoxic effect on a cell, cellular component or biomolecule. Preferably, the reactive species is oxygen. As used herein, a "photosensitizer composition" comprises a photosensitizer coupled to a macromolecular carrier. A "macromolecular carrier" refers to a biomolecule with targeting specificity for one or more components comprising the vulnerable plaque.

In yet another aspect, the present invention comprises methods to detect and/or identify vulnerable plaques by targeting fluorescent compositions, including photosensitizers, fluorescent dyes, and photoactive dyes, to the inflammatory components comprising vulnerable plaques. In one embodiment, a method of detecting a vulnerable plaque in a subject comprises the steps of:

- a) administering a fluorescent composition; and
 - b) localizing the composition to the vulnerable plaque; and
 - c) light activating the composition to illuminate the vulnerable plaque; and
- identifying the vulnerable plaque.

As used herein, a "fluorescent composition" comprises a photosensitizer, fluorescent dye or photoactive dye coupled to a macromolecular carrier. As used herein, the term "fluorescent dye" refers to dyes that are fluorescent when illuminated with light but do not produce reactive species that are phototoxic or otherwise capable of reacting with biomolecules. A photosensitizer will fluoresce when illuminated with a certain wavelength and power of light and also produce reactive species that is phototoxic under the same or different wavelength and power of light. The term "photoactive dye," as used herein, means that the illuminated photosensitizer produces a fluorescent species, but not necessarily a reactive species in phototoxic amounts (i.e., a phototoxic species). Depending on the wavelength and power of light administered, a photosensitizer can be activated to fluoresce and, therefore, act as a photoactive dye, but not produce a phototoxic species. The wavelength and power of light can be adapted by methods known to those skilled in the art to bring about a phototoxic effect where desired.

In yet another embodiment, a method of detecting a vulnerable plaque in a subject comprises the steps of:

- a) administering a detectable amount of at least one fluorescent composition, wherein the fluorescent composition is localized to a vulnerable plaque; and
- b) light activating the vulnerable plaque to produce a fluorescent species; and
- c) identifying the vulnerable plaque.

In yet another aspect, methods of the present invention comprise a combination of detection and treatment. In one embodiment, a method of detecting and treating a vulnerable plaque in a subject comprises the steps of:

- a) administering a detectable amount of at least one fluorescent composition, wherein the fluorescent composition is localized to a vulnerable plaque; and
- b) administering a therapeutically effective amount of at least one photosensitizer composition, wherein the photosensitizer composition is localized to a vulnerable plaque; and
- c) light activating the vulnerable plaque to produce a fluorescent species; and
- d) identifying the vulnerable plaque; and
- e) light activating the photosensitizer composition at the site of the vulnerable plaque to produce a phototoxic species; and
- f) stabilizing the vulnerable plaque against rupture.

In yet another embodiment, a method of detecting and treating a vulnerable plaque in a subject comprises the steps of:

- a) administering a fluorescent composition comprising a photosensitizer coupled to a macromolecular carrier; and
- b) localizing the composition to the vulnerable plaque; and
- c) light activating the composition to illuminate the vulnerable plaque; and
- d) identifying the vulnerable plaque; and

- e) light activating the photosensitizer at the site of the vulnerable plaque to produce a phototoxic species; and
- f) stabilizing the vulnerable plaque against rupture.

In yet another embodiment, a method of detecting and treating a vulnerable plaque in a subject comprises the steps of:

- a) administering a fluorescent composition comprising a photoactive dye coupled to a macromolecular carrier; and
- b) localizing the composition to the vulnerable plaque; and
- c) first light activating the composition to illuminate the vulnerable plaque; and identifying the vulnerable plaque; and
- d) second light activating the photoactive dye at the site of the vulnerable plaque to produce a phototoxic species; and
- e) stabilizing the vulnerable plaque against rupture.

In yet another embodiment, a method of detecting and treating a vulnerable plaque in a subject comprises the steps of:

- a) administering a detectable amount of at least one fluorescent composition comprising a photosensitizer coupled to a macromolecular carrier, wherein the fluorescent composition is localized to a vulnerable plaque; and
- c) light activating the vulnerable plaque to produce a fluorescent species; and
- c) identifying the vulnerable plaque; and
- d) light activating the photosensitizer at the site of the vulnerable plaque to produce a phototoxic species; and
- e) stabilizing the vulnerable plaque against rupture.

In yet another embodiment, a method of detecting and treating a vulnerable plaque in a subject comprises the steps of:

- a) administering a detectable amount of at least one fluorescent composition comprising a photoactive dye coupled to a macromolecular carrier, wherein the fluorescent composition is localized to a vulnerable plaque; and

- b) first light activating the vulnerable plaque to produce a fluorescent species; and
- c) identifying the vulnerable plaque; and
- d) second light activating the photoactive dye at the site of the vulnerable plaque to produce a phototoxic species; and
- e) stabilizing the vulnerable plaque against rupture.

In yet another embodiment, a method of detecting and treating a vulnerable plaque in a subject comprises the steps of:

- a) administering a detectable amount of at least one fluorescent composition, wherein the fluorescent composition is localized to a vulnerable plaque; and administering a therapeutically effective amount of at least one photosensitizer composition, wherein the photosensitizer composition is localized to a vulnerable plaque; and
- b) light activating the vulnerable plaque to produce a fluorescent species; and
- c) identifying the vulnerable plaque; and
- d) light activating the photosensitizer composition at the site of the vulnerable plaque to produce a phototoxic species; and stabilizing the vulnerable plaque against rupture.

Photosensitizer Compositions

Photosensitizers of the present invention can be any known in the art, including, but not limited to, photofrin.RTM, synthetic diporphyrins and dichlorins, phthalocyanines with or without metal substituents, chloroaluminum phthalocyanine with or without varying substituents, chloroaluminum sulfonated phthalocyanine, O-substituted tetraphenyl porphyrins, 3,1-meso tetrakis (o-propionamido phenyl) porphyrin, verdins, purpurins, tin and zinc derivatives of octaethylpurpurin, etiopurpurin, hydroporphyrins, bacteriochlorins of the tetra(hydroxyphenyl) porphyrin series, chlorins, chlorin e₆, mono-l-aspartyl derivative of chlorin e₆, di-l-aspartyl derivative of chlorin e₆, tin(IV) chlorin e₆, meta-tetrahydroxyphenylchlorin, benzoporphyrin derivatives, benzoporphyrin monoacid derivatives, tetracyanoethylene

adducts of benzoporphyrin, dimethyl acetylenedicarboxylate adducts of benzoporphyrin, Diels-Adler adducts, monoacid ring "a" derivative of benzoporphyrin, sulfonated aluminum PC, sulfonated AlPc, disulfonated, tetrasulfonated derivative, sulfonated aluminum naphthalocyanines, naphthalocyanines with or without metal substituents and with or without varying substituents, zinc naphthalocyanine, anthracenediones, anthrapyrazoles, aminoanthraquinone, phenoxazine dyes, phenothiazine derivatives, chalcogenapyrylium dyes, cationic seleno and tellurapyrylium derivatives, ring-substituted cationic PC, pheophorbide derivative, pheophorbide alpha and ether or ester derivatives, pyropheophorbides and ether or ester derivatives, naturally occurring porphyrins, hematoporphyrin, hematoporphyrin derivatives, hematoporphyrin esters or ethers, protoporphyrin, ALA-induced protoporphyrin IX, endogenous metabolic precursors, 5-aminolevulinic acid benzonaphthoporphyrazines, cationic imminium salts, tetracyclines, lutetium texaphyrin, tin-etio-purpurin, porphycenes, benzophenothiazinium, pentaphyrins, texaphyrins and hexaphyrins, 5-amino levulinic acid, hypericin, pseudohypericin, hypocrellin, terthiophenes, azaporphyrins, azachlorins, rose bengal, phloxine B, erythrosine, iodinated or brominated derivatives of fluorescein, merocyanines, nile blue derivatives, pheophytin and chlorophyll derivatives, bacteriochlorin and bacteriochlorophyll derivatives, porphocyanines, benzochlorins and oxobenzochlorins, sapphyrins, oxasapphyrins, cercosporins and related fungal metabolites and combinations thereof.

Several photosensitizers known in the art are FDA approved and commercially available. In a preferred embodiment, the photosensitizer is a benzoporphyrin derivative ("BPD"), such as BPD-MA, also commercially known as BPD Verteporfin or "BPD" (available from QLT). U.S. Patent No. 4,883,790 describes BPD compositions. BPD is a second-generation compound, which lacks the prolonged cutaneous phototoxicity of Photofrin[®] (Levy (1994) *Semin Oncol* 21: 4-10). BPD has been thoroughly characterized (Richter et al., (1987) *JNCI* 79:1327-1331), (Aveline et al. (1994) *Photochem Photobiol* 59:328-35), and it has been found to be a highly potent photosensitizer for PDT. BPD tends to accumulate within atheromatous plaques.

Targeting BPD the inflammatory cells comprising vulnerable plaques according to methods of the present invention will increase the specificity of photoactivation.

Photosensitizers known as texaphyrins also tend to accumulate within atherosclerotic plaques. Targeting texaphyrins to the inflammatory cells comprising vulnerable plaques according to methods of the present invention will increase the specificity of photoactivation. In a preferred embodiment, the photosensitizer is a texaphyrin photosensitizer, such as motexafin lutetium, commercially known as Antrin (available from Pharmacyclics, Hayse et al., (2001) *Cardiovasc. Res.*, 2:449-55).

In a preferred embodiment, the photosensitizer is tin ethyl etiopurpurin, commercially known as purlytin (available from Miravant).

Fluorescent Compositions

Fluorescent compositions of the present invention can be any known in the art, including photosensitizers, fluorescent dyes, and photoactive dyes.

The photosensitizers used for detection of vulnerable plaques can be any known in the art, as previously described. For example, hematoporphyrin derivatives have been used as fluorescent probes to investigate the development of human atherosclerotic plaques (Spokojny (1986) *J. Am. Coll. Cardiol.* 8:1387-1392). Hematoporphyrin derivatives can be used for the detection of vulnerable plaques, particularly plaques with extensive angiogenesis (i.e., new vasa vasorum are leaky, which will prompt accumulation of the hematoporphyrin in the plaque in addition to the selective targeting provided by the macromolecular carrier).

Fluorescent dyes of the present invention can be any known in the art, including, but not limited to 6-carboxy-4',5'-dichloro-2', 7'-dimethoxyfluorescein succinimidyl ester; 5-(and-6)-carboxyeosin; 5-carboxyfluorescein; 6-carboxyfluorescein; 5-(and-6)-carboxyfluorescein; 5-carboxyfluorescein-bis-(5- carboxymethoxy-2-nitrobenzyl) ether, -alanine-carboxamide, or succinimidyl ester; 5-carboxyfluorescein succinimidyl ester; 6-carboxyfluorescein succinimidyl ester; 5-(and-6)-carboxyfluorescein succinimidyl ester; 5-(4,6-dichlorotriazinyl) aminofluorescein; 2',7'-difluorofluorescein; eosin-5-isothiocyanate; erythrosin-5-isothiocyanate; 6-(fluorescein-5-carboxamido) hexanoic acid or succinimidyl ester; 6-(fluorescein-5-(and-6)-carboxamido) hexanoic acid or succinimidyl ester; fluorescein-5-EX succinimidyl ester; fluorescein-5-isothiocyanate;

fluorescein-6-isothiocyanate; Oregon Green® 488 carboxylic acid, or succinimidyl ester; Oregon Green® 488 isothiocyanate; Oregon Green® 488-X succinimidyl ester; Oregon Green® 500 carboxylic acid; Oregon Green® 500 carboxylic acid, succinimidyl ester or triethylammonium salt; Oregon Green® 514 carboxylic acid; Oregon Green® 514 carboxylic acid or succinimidyl ester; Rhodamine Green™ carboxylic acid, succinimidyl ester or hydrochloride; Rhodamine Green™ carboxylic acid, trifluoroacetamide or succinimidyl ester; Rhodamine Green™-X succinimidyl ester or hydrochloride; Rhodol Green™ carboxylic acid, N,O-bis-(trifluoroacetyl) or succinimidyl ester; bis-(4-carboxypiperidiny) sulfonerhodamine or di(succinimidyl ester); 5-(and-6)-carboxynaphthofluorescein, 5-(and-6)-carboxynaphthofluorescein succinimidyl ester; 5-carboxyrhodamine 6G hydrochloride; 6-carboxyrhodamine 6G hydrochloride, 5-carboxyrhodamine 6G succinimidyl ester; 6-carboxyrhodamine 6G succinimidyl ester; 5-(and-6)-carboxyrhodamine 6G succinimidyl ester; 5-carboxy-2',4',5',7'-tetrabromosulfonefluorescein succinimidyl ester or bis-(diisopropylethylammonium) salt; 5-carboxytetramethylrhodamine; 6-carboxytetramethylrhodamine; 5-(and-6)-carboxytetramethylrhodamine; 5-carboxytetramethylrhodamine succinimidyl ester; 6-carboxytetramethylrhodamine succinimidyl ester; 5-(and-6)-carboxytetramethylrhodamine succinimidyl ester; 6-carboxy-X-rhodamine; 5-carboxy-X-rhodamine succinimidyl ester; 6-carboxy-X-rhodamine succinimidyl ester; 5-(and-6)-carboxy-X-rhodamine succinimidyl ester; 5-carboxy-X-rhodamine triethylammonium salt; Lissamine™ rhodamine B sulfonyl chloride; malachite green isothiocyanate; NANOGOLD® mono(sulfosuccinimidyl ester); QSY® 21 carboxylic acid or succinimidyl ester; QSY® 7 carboxylic acid or succinimidyl ester; Rhodamine Red™-X succinimidyl ester; 6-(tetramethylrhodamine-5-(and-6)-carboxamido)hexanoic acid succinimidyl ester; tetramethylrhodamine-5-isothiocyanate; tetramethylrhodamine-6-isothiocyanate; tetramethylrhodamine-5-(and-6)-isothiocyanate; Texas Red® sulfonyl; Texas Red® sulfonyl chloride; Texas Red®-X STP ester or sodium salt; Texas Red®-X succinimidyl ester; Texas Red®-X succinimidyl ester; and X-rhodamine-5-(and-6)-isothiocyanate.

Fluorescent dyes of the present invention can be, for example, bodipy dyes commercially available from Molecular Probes, including, but not limited to BODIPY®

FL; BODIPY® TMR STP ester; BODIPY® TR-X STP ester; BODIPY® 630/650-X STP ester; BODIPY® 650/665-X STP ester; 6-dibromo-4,4-difluoro-5, 7-dimethyl-4-bora-3a,4a-diaza- s-indacene-3-propionic acid succinimidyl ester; 4,4-difluoro-4-bora-3a,4a- diaza-s-indacene-3,5-dipropionic acid; 4,4-difluoro-5,7-dimethyl- 4-bora-3a,4a-diaza-s-indacene- 3-pentanoic acid; 4,4-difluoro-5,7-dimethyl- 4-bora-3a,4a-diaza-s-indacene- 3-pentanoic acid succinimidyl ester; 4,4-difluoro-5,7-dimethyl- 4-bora-3a,4a-diaza-s-indacene- 3-propionic acid; 4,4-difluoro-5,7-dimethyl- 4-bora-3a,4a-diaza-s-indacene- 3-propionic acid succinimidyl ester; 4,4-difluoro-5,7-dimethyl- 4-bora-3a,4a-diaza-s-indacene- 3-propionic acid sulfosuccinimidyl ester or sodium salt; 6-((4,4-difluoro-5,7-dimethyl- 4-bora-3a,4a-diaza-s-indacene-3-propionyl)amino)hexanoic acid; 6-((4,4-difluoro-5,7-dimethyl- 4-bora-3a,4a-diaza-s-indacene-3-propionyl)amino)hexanoic acid or succinimidyl ester; N-(4,4-difluoro-5,7-dimethyl- 4-bora-3a,4a-diaza-s-indacene- 3-propionyl)cysteic acid, succinimidyl ester or triethylammonium salt; 6-4,4-difluoro-1,3-dimethyl- 5-(4-methoxyphenyl)-4-bora-3a,4a 4,4-difluoro-5,7-diphenyl- 4-bora-3a,4a-diaza-s-indacene- 3-propionic acid; 4,4-difluoro-5,7-diphenyl- 4-bora-3a,4a-diaza-s-indacene- 3-propionic acid succinimidyl ester; 4,4-difluoro-5-phenyl-4-bora- 3a,4a-diaza-s-indacene-3-propionic acid succinimidyl ester; 6-((4,4-difluoro-5-phenyl- 4-bora-3a,4a-diaza-s-indacene- 3-propionyl)amino)hexanoic acid or succinimidyl ester; 4,4-difluoro-5-(4-phenyl-1, 3-butadienyl)-4-bora-3a,4a- diaza-s-indacene-3-propionic acid succinimidyl ester; 4,4-difluoro-5-(2-pyrrolyl) -4-bora-3a,4a-diaza-s-indacene- 3-propionic acid succinimidyl ester; 6-(((4,4-difluoro-5-(2-pyrrolyl) -4-bora-3a,4a-diaza-s-indacene- 3-yl)styryloxy)acetyl)aminohexanoic acid or succinimidyl ester; 4,4-difluoro-5-styryl-4-bora- 3a,4a-diaza-s-indacene-3-propionic acid; 4,4-difluoro-5-styryl-4-bora- 3a,4a-diaza-s-indacene-3-propionic acid succinimidyl ester; 4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene- 8-propionic acid; 4,4-difluoro-1,3,5,7-tetramethyl- 4-bora-3a,4a-diaza-s-indacene- 8-propionic acid succinimidyl ester; 4,4-difluoro-5-(2-thienyl) -4-bora-3a,4a-diaza-s-indacene- 3-propionic acid succinimidyl ester; 6-(((4,4-difluoro-5-(2- thienyl)-4-bora-3a,4a-diaza s-indacene-3-yl)phenoxy)acetyl) amino)hexanoic acid or succinimidyl ester; and 6-(((4,4-difluoro-5-(2-thienyl) -4-bora-3a,4a-diaza-s-indacene-3-yl)styryloxy)acetyl)aminohexanoic acid or succinimidyl ester.

Fluorescent dyes the present invention can be, for example, alexa fluor dyes commercially available from Molecular Probes, including but not limited to Alexa Fluor® 350 carboxylic acid; Alexa Fluor® 430 carboxylic acid; Alexa Fluor® 488 carboxylic acid; Alexa Fluor® 532 carboxylic acid; Alexa Fluor® 546 carboxylic acid; Alexa Fluor® 555 carboxylic acid; Alexa Fluor® 568 carboxylic acid; Alexa Fluor® 594 carboxylic acid; Alexa Fluor® 633 carboxylic acid; Alexa Fluor® 647 carboxylic acid; Alexa Fluor® 660 carboxylic acid; and Alexa Fluor® 680 carboxylic acid.

Fluorescent dyes the present invention can be, for example, cy dyes commercially available from Amersham-Pharmacia Biotech, including, but not limited to Cy3 NHS ester; Cy 5 NHS ester; Cy5.5 NHS ester; and Cy 7 NHS ester.

Photoactive dyes of the present invention can be any photosensitizer known in the art which will fluoresce but not necessarily produce a reactive species in phototoxic amounts when illuminated. Depending on the wavelength and power of light administered, a photosensitizer can be activated to fluoresce and, therefore, act as a photoactive dye, but not produce a phototoxic effect unless, in some cases, the wavelength and power of light is suitably adapted to induce a phototoxic effect.

Photosensitizer Composition Targeting

Selectivity of photosensitizers for target tissues of the present invention is achieved by using covalent conjugates, or non-covalent complexes between photosensitizers and macromolecular carriers with targeting specificity for one or more components comprising the vulnerable plaque (Hasan, T. (1992) *In*: B. Henderson and T. Dougherty (eds.), *Photodynamic Therapy: Basic Principles and Clinical Applications*, pp. 187-200: Marcel Dekker). Accordingly, photosensitizer compositions of the present invention comprise one or more photosensitizers and/or macromolecular carriers. Use of macromolecular carriers advantageously allows the photosensitizer to be selected according to optical and photophysical properties, without relying on the molecular structure of the photosensitizer to provide a tissue-targeting effect.

Generally, macromolecular targeting is based on two facets of molecular structure. Firstly features of the macromolecules such as size, charge, hydrophobicity and biodegradability can be manipulated to increase accumulation or retention in the plaque, and, secondly, the macromolecular conjugate can be designed to recognize

antigens, receptors or other cell type specific structures present on inflammatory cells. In a preferred embodiment, the macromolecular carrier is selected from the group consisting of serum proteins including receptor ligands (Hamblin et al. (1994) J. Photochem. Photobiol. 26:147-157; Hamblin and Newman (1994) J. Photochem. Photobiol. 26:45-56), microspheres (Bachor et al. (1991) Proc. Natl. Acad. Sci. U.S.A. 88:1580-1584), liposomes (Polo et al. (1996) Cancer Lett. 109:57-61), polymers (Hamblin et al. (1999) Br. J. Cancer 81:261-268), monoclonal antibodies (Hamblin et al. (2000) Br. J. Cancer 83:1544-1551), growth factors (Gijssens and De Witte (1998) Int. J. Oncol. 13:1171-1177), peptides (Krinick, (1994) J. Biomater. Sci. Polym. Ed. 5: 303-324), hormones (Akhlynina et al. (1995) Cancer Res. 55:1014-1019) and lipoproteins (Schmidt-Erfurth et al. (1997) Br. J. Cancer 75:54-61).

In a preferred embodiment, photosensitizer compositions of the present invention are coupled to macromolecular carrier comprising ligands that bind to "scavenger receptors." Scavenger receptors are membrane proteins expressed on the surface of macrophages, monocytes, endothelial cells and smooth muscle cells that recognize a wide range of ligands, both naturally occurring and synthetic (Freeman et al. (1997) Curr. Opin. Hematol. 4:41-47). Presently, there are six members of the scavenger receptor family belonging to three classes (e.g., class A, B or C). After initial binding to the scavenger receptor, the ligands are rapidly internalized and are routed to lysosomes for degradation by proteases and other lysosomal enzymes. The wide and diverse range of structures recognized by these receptors has led to them being termed "molecular flypaper" (Krieger et al. (1992) Trends Biochem. Sci. 17:141-146, 1992). The ligands are all macromolecules with a pronounced anionic charge that have some common conformational features (Haberland and Fogelman (1985) Proc. Natl. Acad. Sci. U.S.A. 82:2693-2697; Takata (1989) Biochem. Biophys. Acta. 984:273-280). Specific targeting of compositions to J774 and other macrophage-like cells *in vitro* has been achieved with conjugates of maleylated albumin, daunorubicin and doxorubicin (Mukhopadhyay et al (1992) Biochem J. 284:237-241; Basu et al. (1994) FEBS Lett. 342:249-254; Hamblin et al. (2000) Photochem Photobiol. 4:533-540).

Numerous scavenger receptor ligands known in the art (either with or without polyethyl glycolization) can be used to target photosensitizer compositions of the

present invention to vulnerable plaques, including, but not limited to maleylated albumin, oxidized low density lipoprotein, acetylated low density lipoprotein, oxidized high density lipoprotein, malondialdehyde treated proteins, lipotechoic acid, formaldehyde treated albumin, glycated albumin, polyinosinic acid, glycated lipoproteins, dextran sulfate, anionic phospholipids (phosphatidyl serine), fucoidin, carrageenan, polyvinyl sulfate, monoclonal antibodies that recognize CD11b or c, CD13, CD14, CD16a, CD32 or CD68.

In a preferred embodiment, photosensitizer compositions of the present invention are coupled to macromolecular carriers that target macrophages and/or monocytes of vulnerable plaques. These macromolecular carriers can be targeted to, for example, including, tenascin C, tissue factor, tissue inhibitor of MMP 1 and 2, oxidized LDL receptor (also known in the art as CD36), heme oxygenase-1, human cartilage gp-39, IL-6, IL-6 receptor, IL-10, IL-10 receptor, lectin-like oxidized LDL-receptor ("LOX-1"), monocyte inflammatory protein-1 and receptors thereof, and macrophage chemoattractant protein-1 receptor ("CCR-5"). Such macromolecular carriers can be, for example, antibodies against these biomolecules, ligands binding the same or analogs thereof.

In a preferred embodiment, photosensitizer compositions of the present invention are coupled to macromolecular carriers that target T cells of vulnerable plaques. These macromolecular carriers can be targeted to, for example, IL-10, IL-10 receptor, monocyte inflammatory protein-1 and receptors thereof and transferrin. Such macromolecular carriers can be, for example, antibodies against these biomolecules, ligands binding the same or analogs thereof, including, but not limited to monoclonal antibodies that recognize CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD25, CD28, CD44, CD71 or transferrin.

In a preferred embodiment, photosensitizer compositions of the present invention are delivered via macromolecular carriers that target the lipid pool of the atheroma, including but not limited to hydrophobic photosensitizers or photosensitizers delivered in hydrophobic vehicles such as liposomes (with positive, neutral or negatively charged and optionally containing cholesterol or cardiolipin) cremaphor EL,

PEG/solvent mixtures, iodized castor oil, and various nanoparticles and micellar preparations.

In a preferred embodiment, photosensitizer compositions of the present invention are coupled to macromolecular carriers that target proteases that degrade extracellular matrix (e.g., metalloproteinases), including but not limited to monoclonal antibodies against the protease and proteinase substrates.

In a preferred embodiment, photosensitizer compositions of the present invention are coupled to macromolecular carriers that target the endothelial cells of vulnerable plaques. These macromolecular carriers can be targeted to, for example, endothelial adhesion molecules including, but not limited to, ICAM (also known in the art as CD54) and VCAM (also known in the art as CD106), angiotensin II, angiotensin converting enzyme (also known in the art as CD143), endothelial derived lipase, tissue factor, heme oxygenase-1, LOX-1, low density lipoprotein ("LDL"), high density lipoprotein, ("HDL"), P-selectin, L-selectin and E-selectin. Such macromolecular carriers can be, for example, antibodies against these biomolecules, ligands binding the same or analogs thereof.

In a preferred embodiment, photosensitizer compositions of the present invention are coupled to macromolecular carriers that target neutrophils of vulnerable plaques. These macromolecular carriers can be targeted to, for example, myeloperoxidase. Such macromolecular carriers can be, for example, antibodies against these biomolecules, ligands binding the same or analogs thereof.

In a preferred embodiment, photosensitizer compositions of the present invention are coupled to macromolecular carriers that target B cells of vulnerable plaques. These macromolecular carriers can be targeted to, for example, IL-6, IL-6 receptor, IL-10 and IL-10 receptor. Such macromolecular carriers can be, for example, antibodies against these biomolecules, ligands binding the same or analogs thereof.

In a preferred embodiment, photosensitizer compositions of the present invention are coupled to macromolecular carriers that target smooth muscle cells of vulnerable plaques. These macromolecular carriers can be targeted to, for example, LOX-1. Such macromolecular carriers can be, for example, antibodies against these biomolecules, ligands binding the same or analogs thereof.

The features of a vulnerable plaque that are distinguishable from stable atheromatous plaques advantageously distinguish vulnerable plaques from stable atheromatous plaques according to methods of the present invention. Vulnerable plaques comprise an abundance of inflammatory cells, a large lipid pool, and a thin fibrous cap. Preferably, a vulnerable plaque comprises a fibrous cap that is less than about 150 microns thick. More preferably, a vulnerable plaque comprises a fibrous cap that is less than about 100 microns thick (e.g., between about 60 and 100 microns thick). Preferably, a vulnerable plaque comprises a macrophage and/or monocyte content that is greater than about 10%. More preferably, a vulnerable plaque comprises a macrophage and/or monocyte content that is greater than about 25%. Preferably, a vulnerable plaque comprises a lipid content that is greater than about 10%. More preferably, a vulnerable plaque comprises a lipid content that is greater than about 25%. Thus, targeting a photosensitizer or fluorescent composition to activated macrophages or proteases that degrade extracellular matrix via a macromolecular carrier, for example, confers a selective advantage on a vulnerable plaque such that uptake of the composition is far greater than in a non-vulnerable plaque. Thus, photodetection or photoactivation of the vulnerable plaque can be carried out at a wavelength and power of light that has an insubstantial or negligible effect on non-vulnerable plaques. Thus, the methods and devices of the present invention are advantageously suited for detection and therapy of vulnerable plaques and not merely commonplace stable atheromatous plaques.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook, 1989); "Oligonucleotide Synthesis" (Gait, 1984); "Animal Cell Culture" (Freshney, 1987); "Methods in Enzymology" "Handbook of Experimental Immunology" (Weir, 1996); "Gene Transfer Vectors for Mammalian Cells" (Miller and Calos, 1987); "Current Protocols in Molecular Biology" (Ausubel, 1987); "PCR: The Polymerase Chain Reaction", (Mullis, 1994); "Current Protocols in Immunology" (Coligan, 1991). These techniques

are applicable to the production of the polynucleotides and polypeptides of the invention, and, as such, may be considered in making and practicing the invention. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

The term "coupling agent" as used herein, refers to a reagent capable of coupling a photosensitizer to a macromolecular carrier, or a photosensitizer or a macromolecular carrier to a "backbone" or "bridge" moiety. Any bond which is capable of linking the components such that they are stable under physiological conditions for the time needed for administration and treatment is suitable, but covalent linkages are preferred. The link between two components may be direct, e.g., where a photosensitizer is linked directly to a macromolecular carrier, or indirect, e.g., where a photosensitizer is linked to an intermediate, e.g., linked to a backbone, and that intermediate being linked to the macromolecular carrier. A coupling agent should function under conditions of temperature, pH, salt, solvent system, and other reactants that substantially retain the chemical stability of the photosensitizer, the backbone (if present), and the macromolecular carrier.

A coupling agent is not always required, for example, where the fluorescent compound is in the form of a sulfonyl chloride, isothiocyanate or succinimidyl ester, no coupling agent is necessary.

A coupling agent can link components without the addition to the linked components of elements of the coupling agent. Other coupling agents result in the addition of elements of the coupling agent to the linked components. For example, coupling agents can be cross-linking agents that are homo- or hetero-bifunctional, and wherein one or more atomic components of the agent can be retained in the composition. A coupling agent that is not a cross-linking agent can be removed entirely during the coupling reaction, so that the molecular product can be composed entirely of the photosensitizer, the targeting moiety, and a backbone moiety (if present).

Many coupling agents react with an amine and a carboxylate, to form an amide, or an alcohol and a carboxylate to form an ester. Coupling agents are known in the art, see, e.g., M. Bodansky, "Principles of Peptide Synthesis", 2nd ed., referenced herein, and T. Greene and P. Wuts, "Protective Groups in Organic Synthesis," 2nd Ed, 1991,

John Wiley, NY. Coupling agents should link component moieties stably, but such that there is only minimal or no denaturation or deactivation of the photosensitizer or the macromolecular carrier.

The photosensitizer conjugates of the invention can be prepared by coupling the photosensitizer to macromolecular carriers using methods described in the following Examples, or by methods known in the art. A variety of coupling agents, including cross-linking agents, can be used for covalent conjugation. Examples of cross-linking agents include N,N'-dicyclohexylcarbodiimide (DCC), N-succinimidyl-S-acetylthioacetate (SATA), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), ortho-phenylenedimaleimide (o-PDM), and sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) (Karpovsky et al. (1984) J. Exp. Med. 160:1686; Liu, MA et al. (1985) Proc. Natl. Acad. Sci. USA 82:8648). Other methods include those described by Paulus and Behring (1985) Ins. Mitt., 78:118-132; Brennan et al. (1985) Science 229:81-83 and Glennie et al., (1987) J. Immunol, 139:2367-2375. A large number of coupling agents for peptides and proteins, along with buffers, solvents, and methods of use, are described in the Pierce Chemical Co. catalog, pages T155 -T-200, 1994 (3747 N. Meridian Rd., Rockford IL, 61105, U.S.A.; Pierce Europe B.V., P.O. Box 1512, 3260 BA Oud Beijerland, The Netherlands), the contents of which are hereby incorporated by reference.

DCC is a useful coupling agent (Pierce #20320; Rockland, IL). It promotes coupling of the alcohol NHS to chlorin e6 in DMSO (Pierce #20684), forming an activated ester which can be cross-linked to polylysine. DCC (N,N'-dicyclohexylcarbodiimide) is a carboxy-reactive cross-linker commonly used as a coupling agent in peptide synthesis, and has a molecular weight of 206.32. Another useful cross-linking agent is SPDP (Pierce #21557), a heterobifunctional cross-linker for use with primary amines and sulfhydryl groups. SPDP has a molecular weight of 312.4, a spacer arm length of 6.8 angstroms, is reactive to NHS-esters and pyridyldithio groups, and produces cleavable cross-linking such that, upon further reaction, the agent is eliminated so the photosensitizer can be linked directly to a backbone or macromolecular carrier. Other useful conjugating agents are SATA (Pierce #26102) for introduction of blocked SH groups for two-step cross-linking, which is deblocked with

hydroxylamine-25-HCl (Pierce #26103), and sulfo-SMCC (Pierce #22322), reactive towards amines and sulfhydryls. Other cross-linking and coupling agents are also available from Pierce Chemical Co. (Rockford, IL). Additional compounds and processes, particularly those involving a Schiff base as an intermediate, for conjugation of proteins to other proteins or to other compositions, for example to reporter groups or to chelators for metal ion labeling of a protein, are disclosed in EPO 243,929 A2 (published Nov. 4, 1987).

Photosensitizers which contain carboxyl groups can be joined to lysine ϵ -amino groups in the target polypeptides either by preformed reactive esters (such as N-hydroxy succinimide ester) or esters conjugated in situ by a carbodiimide-mediated reaction. The same applies to photosensitizers that contain sulfonic acid groups, which can be transformed to sulfonyl chlorides, which react with amino groups. Photosensitizers that have carboxyl groups can be joined to amino groups on the polypeptide by an in situ carbodiimide method. Photosensitizers can also be attached to hydroxyl groups, of serine or threonine residues or to sulfhydryl groups, of serine or threonine residues or to sulfhydryl groups of cysteine residues.

Methods of joining components of a conjugate, e.g., coupling polyamino acid chains bearing photosensitizers to antibacterial polypeptides, can use heterobifunctional cross linking reagents. These agents bind a functional group in one chain and to a different functional group in the second chain. These functional groups typically are amino, carboxyl, sulfhydryl, and aldehyde. There are many permutations of appropriate moieties that will react with these groups and with differently formulated structures, to conjugate them together (described in the Pierce Catalog and Merrifield et al. (1994) Ciba Found Symp. 186:5-20).

The production and purification of photosensitizers coupled to macromolecular carriers can be practiced by methods known in the art. Yield from coupling reactions can be assessed by spectroscopy of product eluting from a chromatographic fractionation in the final step of purification. The presence of uncoupled photosensitizer and reaction products containing the photosensitizer can be followed by the physical property that the photosensitizer moiety absorbs light at a characteristic wavelength and extinction coefficient, so incorporation into products can be monitored

by absorbance at that wavelength or a similar wavelength. Coupling of one or more photosensitizer molecules to a macromolecular carrier or to a backbone shifts the peak of absorbance in the elution profile in fractions eluted using sizing gel chromatography, e.g., with the appropriate choice of Sephadex G50, 6100, or 6200 or other such matrices (Pharmacia-Biotech, Piscataway NJ). Choice of appropriate sizing gel, for example Sephadex gel, can be determined by that gel in which the photosensitizer elutes in a fraction beyond the excluded volume of material too large to interact with the bead, i.e., the uncoupled starting photosensitizer composition interacts to some extent with the fractionation bead and is concomitantly retarded to some extent. The correct useful gel can be predicted from the molecular weight of the uncoupled photosensitizer. The successful reaction products of photosensitizer compositions coupled to additional moieties generally have characteristic higher molecular weights, causing them to interact with the chromatographic bead to a lesser extent, and thus appear in fractions eluting earlier than fractions containing the uncoupled photosensitizer substrate. Unreacted photosensitizer substrate generally appears in fractions characteristic of the starting material, and the yield from each reaction can thus be assessed both from size of the peak of larger molecular weight material, and the decrease in the peak of characteristic starting material. The area under the peak of the product fractions is converted to the size of the yield using the molar extinction coefficient.

The product can be analyzed using NMR, integrating areas of appropriate product peaks, to determine relative yields with different coupling agents. A red shift in absorption of a photosensitizer has often been observed following coupling to a polyamino acid. Coupling to a larger carrier such as a protein might produce a comparable shift, as coupling to an antibody resulted in a shift of about 3-5 nm in that direction compared to absorption of the free photosensitizer. Relevant absorption maxima and extinction coefficients in 0.1M NaOH/1% SDS are, for chlorin e6, 400 nm and 150,000 M⁻¹, cm⁻¹, and for benzoporphyrin derivative, 430 nm and 61,000 M⁻¹, cm⁻¹.

Photosensitizers compositions of the invention include those in which a photosensitizer is coupled directly to a macromolecular carrier, such as a scavenger

receptor ligand. Other photosensitizer compositions of the invention include a "backbone" or "bridge" moiety, such as a polyamino acid, in which the backbone is coupled both to a photosensitizer and to a macromolecular carrier.

Inclusion of a backbone in a composition with a photosensitizer and a macromolecular carrier can provide a number of advantages, including the provision of greater stoichiometric ranges of photosensitizer and macromolecular carriers coupled per backbone. If the backbone possesses intrinsic affinity for a target organism, the affinity of the composition can be enhanced by coupling to the backbone. The specific range of organisms that can be targeted with one composition can be expanded by coupling two or more different macromolecular carriers to a single photosensitizer-backbone composition.

Peptides useful in the methods and compounds of the invention for design and characterization of backbone moieties include poly-amino acids which can be homo- and hetero-polymers of L-, D-, racemic DL- or mixed L- and D-amino acid composition, and which can be of defined or random mixed composition and sequence. These peptides can be modeled after particular natural peptides, and optimized by the technique of phage display and selection for enhanced binding to a chosen target, so that the selected peptide of highest affinity is characterized and then produced synthetically. Further modifications of functional groups can be introduced for purposes, for example, of increased solubility, decreased aggregation, and altered extent of hydrophobicity. Examples of nonpeptide backbones include nucleic acids and derivatives of nucleic acids such as DNA, RNA and peptide nucleic acids; polysaccharides and derivatives such as starch, pectin, chitins, celluloses and hemi-methylated celluloses; lipids such as triglyceride derivatives and cerebroside; synthetic polymers such as polyethylene glycols (PEGs) and PEG star polymers; dextran derivatives, polyvinyl alcohols, N-(2-hydroxypropyl)-methacrylamide copolymers, poly (DL-glycolic acid-lactic acid); and compositions containing elements of any of these classes of compounds.

The affinity of a photosensitizer composition can be refined by modifying the charge of a component of the composition. Conjugates such as poly-L-lysine chlorin e6 can be made in varying sizes and charges (cationic, neutral, and anionic), for example,

free NH₂ groups of the polylysine are capped with acetyl, succinyl, or other R groups to alter the charge of the final composition. Net charge of a composition of the present invention can be determined by isoelectric focusing (IEF). This technique uses applied voltage to generate a pH gradient in a non-sieving acrylamide or agarose gel by the use of a system of ampholytes (synthetic buffering components). When charged polypeptides are applied to the gel they will migrate either to higher pH or to lower pH regions of the gel according to the position at which they become non-charged and hence unable to move further. This position can be determined by reference to the positions of a series of known IEF marker proteins.

Photosensitizer compositions of the present invention can comprise photosensitizers coupled to antibodies, which are known in the art as "photoimmunoconjugates." The antibody component of the photoimmunoconjugate can bind with specificity to an epitope present on the surface of a cell comprising the vulnerable plaque. As used herein, the term "binding with specificity" means that cells that do not express the epitope are only poorly recognized by the antibody.

The term "antibody" as used in this invention includes intact molecules as well as fragments thereof, such as Fab and Fab', which are capable of binding the epitopic determinant. Fab fragments retain an entire light chain, as well as one-half of a heavy chain, with both chains covalently linked by the carboxy terminal disulfide bond. Fab fragments are monovalent with respect to the antigen-binding site. The antibodies of the invention comprise whole native antibodies, bispecific antibodies; chimeric antibodies; Fab, Fab', single chain variable region fragments (scFv) and fusion polypeptides. Preferably, the antibodies of the invention are monoclonal.

The antibodies of this invention can be prepared in several ways. Methods of producing and isolating whole native antibodies, bispecific antibodies; chimeric antibodies; Fab, Fab', single chain V region fragments (scFv) and fusion polypeptides are known in the art. See, for example, Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York (Harlow and Lane, 1988).

Antibodies are most conveniently obtained from hybridoma cells engineered to express an antibody. Methods of making hybridomas are well known in the art. The

hybridoma cells can be cultured in a suitable medium, and spent medium can be used as an antibody source. Polynucleotides encoding the antibody can in turn be obtained from the hybridoma that produces the antibody, and then the antibody may be produced synthetically or recombinantly from these DNA sequences. For the production of large amounts of antibody, it is generally more convenient to obtain an ascites fluid. The method of raising ascites generally comprises injecting hybridoma cells into an immunologically naive histocompatible or immunotolerant mammal, especially a mouse. The mammal may be primed for ascites production by prior administration of a suitable composition, e.g., Pristane.

Another method of obtaining antibodies is to immunize suitable host animals with an antigen and to follow standard procedures for polyclonal or monoclonal production. Monoclonal antibodies (Mabs) thus produced can be "humanized" by methods known in the art. Examples of humanized antibodies are provided, for instance, in United States Patent Nos. 5,530,101 and 5,585,089.

"Humanized" antibodies are antibodies in which at least part of the sequence has been altered from its initial form to render it more like human immunoglobulins. In one version, the heavy chain and light chain C regions are replaced with human sequence. In another version, the CDR regions comprise amino acid sequences for recognition of antigen of interest, while the variable framework regions have also been converted to human sequences. See, for example, EP 0329400. In a third version, variable regions are humanized by designing consensus sequences of human and mouse variable regions, and converting residues outside the CDRs that are different between the consensus sequences. The invention encompasses humanized Mabs.

The invention also encompasses hybrid antibodies, in which one pair of heavy and light chains is obtained from a first antibody, while the other pair of heavy and light chains is obtained from a different second antibody. Such hybrids may also be formed using humanized heavy and light chains.

Construction of phage display libraries for expression of antibodies, particularly the Fab or scFv portion of antibodies, is well known in the art (Heitner et al. (2001) J Immunol Methods 248:17-30). The phage display antibody libraries that express antibodies can be prepared according to the methods described in U.S. Pat. No.

5,223,409 incorporated herein by reference. Procedures of the general methodology can be adapted using the present disclosure to produce antibodies of the present invention. The method for producing a human monoclonal antibody generally involves (1) preparing separate heavy and light chain-encoding gene libraries in cloning vectors using human immunoglobulin genes as a source for the libraries, (2) combining the heavy and light chain encoding gene libraries into a single dicistronic expression vector capable of expressing and assembling a heterodimeric antibody molecule, (3) expressing the assembled heterodimeric antibody molecule on the surface of a filamentous phage particle, (4) isolating the surface-expressed phage particle using immunoaffinity techniques such as panning of phage particles against a preselected antigen, thereby isolating one or more species of phagemid containing particular heavy and light chain-encoding genes and antibody molecules that immunoreact with the preselected antigen.

Single chain variable region fragments are made by linking light and heavy chain variable regions by using a short linking peptide. Any peptide having sufficient flexibility and length can be used as a linker in a scFv. Usually the linker is selected to have little to no immunogenicity. An example of a linking peptide is (GGGS)₃, which bridges approximately 3.5 nm between the carboxy terminus of one variable region and the amino terminus of another variable region. Other linker sequences can also be used. All or any portion of the heavy or light chain can be used in any combination. Typically, the entire variable regions are included in the scFv. For instance, the light chain variable region can be linked to the heavy chain variable region. Alternatively, a portion of the light chain variable region can be linked to the heavy chain variable region, or a portion thereof. Also contemplated are compositions comprising a biphasic scFv could be constructed in which one component is a polypeptide that recognizes an antigen and another component is a different polypeptide that recognizes a different antigen, such as a T cell epitope.

ScFvs can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing a polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or

mammalian cells, or prokaryotic, such as *Escherichia coli*, and the protein expressed by the polynucleotide can be isolated using standard protein purification techniques.

A particularly useful system for the production of scFvs is plasmid pET-22b(+) (Novagen, Madison, WI) in *E. coli*. pET-22b(+) contains a nickel ion binding domain consisting of 6 sequential histidine residues, which allows the expressed protein to be purified on a suitable affinity resin. Another example of a suitable vector is pcDNA3 (Invitrogen, San Diego, CA), described above.

Expression conditions should ensure that the scFv assumes functional and, preferably, optimal tertiary structure. Depending on the plasmid used (especially the activity of the promoter) and the host cell, it may be necessary or useful to modulate the rate of production. For instance, use of a weaker promoter, or expression at lower temperatures, may be necessary or useful to optimize production of properly folded scFv in prokaryotic systems; or, it may be preferable to express scFv in eukaryotic cells. Antibody purification methods may include salt precipitation (for example, with ammonium sulfate), ion exchange chromatography (for example, on a cationic or anionic exchange column run at neutral pH and eluted with step gradients of increasing ionic strength), gel filtration chromatography (including gel filtration HPLC), and chromatography on affinity resins such as protein A, protein G, hydroxyapatite, and anti-immunoglobulin.

Photosensitizers can be linked to antibodies according to any method known in the art. For example, the antibody can be directly linked to the photosensitizer through a polymer or a polypeptide linkage. Polymers of interest include, but are not limited to polyamines, polyethers, polyamine alcohols, derivitized to components by means of ketones, acids, aldehydes, isocyanates or a variety of other groups. Polypeptide linkages can comprise, for example poly-L-lysine linkages (Del Governatore et al.(2000) Br. J. Cancer 82:56-64; Hamblin et al. (2000) Br. J. Cancer 83:1544-41; Molpus et al. (2000) Gynecol Oncol 76:397-404). The antibody can be linked to a photosensitizer and at least one solubilizing agent each of which are independently bound to the antibody through a direct covalent linkage. The direct covalent linkage can be, for example, an amide linkage to a lysine residue of the antibody, as described

in U.S. application serial No. 10/137,029, the contents of which are herein incorporated by reference.

Photosensitizer compositions of the present invention can comprise photosensitizers linked to macromolecular carriers comprising the sequences of naturally occurring proteins and peptides, from variants or fragments of these peptides, and from biologically or chemically synthesized peptides or peptide fragments. Naturally occurring peptides which have affinity for one or more target cells can provide sequences from which additional peptides with desired properties, e.g., increased affinity or specificity, can be synthesized individually or as members of a library of related peptides. Such peptides can be selected on the basis of affinity for the target cell.

The term "or (a) fragment(s) thereof" as employed in the present invention and in context with polypeptides of the invention, comprises specific peptides, amino acid stretches of the polypeptides as disclosed herein. It is preferred that said "fragment(s) thereof" is/are functional fragment(s). The term "functional fragment" denotes a part of the above identified polypeptide of the invention which fulfills, at least in part, physiologically and/or structurally related activities of the polypeptide of the invention. The polypeptides of the present invention can be recombinant polypeptides expressed in eukaryotic cells, like mammalian cells.

Generally, recombinant DNA technology has enabled the expression of foreign (heterologous) proteins in cell lines of choice. In this process, a vector containing genetic material directing a cell to produce a protein encoded by a portion of a heterologous DNA sequence is introduced into the host, and the transformed host cells can be fermented, cultured or otherwise subjected to conditions which facilitate the expression of the heterologous DNA, leading to the formation of large quantities of the desired protein. Plasmids are extensively used as vectors to clone DNA molecules. Most plasmid vectors are made by taking DNA from a variety of replicons (plasmids, bacteriophage chromosomes and bacterial chromosomes) and joining the DNA together (using restriction enzymes and DNA ligase) to form a plasmid that has an origin of replication, a selection marker (usually an antibiotic-resistance gene) and a promoter for expressing genes of interest in the required host cell. A vector can be, for example, as

in U.S. Patent Nos. 5,990,091 and 6,004,777, and as in PCT/US00/04203. Methods for generation and use of recombinant vectors *in vitro* are well known in the art. See Sambrook, Fritsch and Maniatis, Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, 1989 (e.g., procedures for isolating DNA, constructing recombinant vectors, transfecting and transforming cells and producing heterologous peptides).

Furthermore, the recombinant vector can, in addition to the nucleic acid sequences of the invention (e.g., those encoding the targeting peptide or functional fragments thereof), comprise expression control elements, allowing proper expression of the coding regions in suitable hosts. Such control elements are known in the art and can include a promoter, a splice cassette, translation initiation codon, translation and insertion site for introducing an insert into the vector. Preferably, the nucleic acid molecule is operatively linked to expression control sequences allowing expression in eukaryotic or prokaryotic cells.

Control elements ensuring expression in eukaryotic and prokaryotic cells are well known to those skilled in the art. As mentioned herein above, they usually comprise regulatory sequences ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements can include transcriptional as well as translational enhancers, and/or naturally-associated or heterologous promoter regions. Possible regulatory elements permitting expression in for example mammalian cells comprise the CMV- HSV thymidine kinase promoter, SV40, RSV-promoter (Rous sarcoma virus), human elongation factor 1 α -promoter, aPM-I promoter (Schaffer et al. (1999) Biochem. Biophys. Res. Commun. 260:416-425), or inducible promoter(s), like, metallothionein or tetracycline, or enhancers, like CMV enhancer or SV40-enhancer. For the expression in prokaryotic cells, a multitude of promoters including, for example, the tac-lac-promoter or the trp promoter, has been described. Besides elements that are responsible for the initiation of transcription, such regulatory elements can also comprise transcription termination signals, such as SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia),

pRc/CMV, pcDNA1, pcDNA3 (Invitrogen), pSPORT1 (GIBCO BRL), Casper, Casper-HS43, pUAST, or prokaryotic expression vectors, such as lambda gt11.

Furthermore, depending on the expression system, leader sequences capable of directing the polypeptide to a cellular compartment can be added to the coding sequence of the nucleic acid molecules of the invention and are well known in the art. The leader sequence(s) is assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein, or a protein thereof, into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an C- or N-terminal identification peptide imparting desired characteristics, e.g., stabilization of expressed recombinant products. Once the vector has been incorporated into the appropriate cell line, the cells are maintained under conditions suitable for high level expression of the nucleotide sequences.

A cell can be transfected or transformed with a recombinant vector encoding the targeting peptide of the present invention. Methods of transformation and transfection are well known in the art. The transformed cells can be grown in fermentors and cultured according to techniques known in the art to achieve optimal cell growth. The resulting transformed or transfected cell lines are genetically modified with a nucleic acid molecule according to the invention or with a vector comprising such a nucleic acid molecule. The term "genetically modified" means that the cell comprises in addition to its natural genome a nucleic acid molecule or vector according to the invention which was introduced into the cell or host or into one of its predecessors/parents. The nucleic acid molecule or vector can be present in the genetically modified cell either as an independent molecule outside the genome, preferably as a molecule that is capable of replication, or it can be stably integrated into the genome of the cell.

The present invention can utilize any suitable prokaryotic or eukaryotic cell. Suitable prokaryotic cells are those generally used for cloning like *Escherichia coli* or *Bacillus subtilis*. Eukaryotic cells comprise, for example, fungal or animal cells, and are generally used for conducting the specificity assay. Animal cells are preferably used for conducting the specificity assay. Suitable animal cells are, for instance, insect

cells, vertebrate cells, preferably mammalian cells. Further suitable cell lines known in the art are obtainable from cell line depositories, like the American Type Culture Collection (ATCC) and the AIDS Research and Reference Reagent Program Catalog. Derivation of primary cells from an animal, preferably a mammal, and even more preferable a human, can also be undertaken for the purposes of establishing a suitable cell line.

Detectable compositions of the present invention can further comprise macromolecular carriers that are radiolabeled. For example, photosensitizer compositions of the present invention can comprise radiolabeled macromolecular carriers coupled to photosensitizers. A number of radiolabeled macromolecular carriers have been tested for their ability to bind to and permit scintigraphic detection of atherothrombotic materials. These include labeled antibodies to oxidized LDL, fibrinogen, autologous platelets, fibrin fragment E1, plasminogen activators, and ^{99m}Tc -conjugated antibodies against modified LDL (Tsimikas et al. (1999) J. Nucl. Cardiol. 6: 41-53).

Examples of appropriate radionuclides for use in radiolabeling include: ^{131}I , ^{125}I , ^{123}I , ^{99m}Tc , ^{18}F , ^{68}Ga , ^{67}Ga , ^{72}As , ^{89}Zr , ^{62}Cu , ^{111}Cu , ^{203}In , ^{198}Pb , ^{198}Hg , ^{97}Ru , ^{11}C and ^{201}Tl . Highly specific and sensitive labels are provided by radionuclides, which can then be detected using positron emission tomography (PET) or Single Photon Emission Computed Tomography (SPECT) imaging. Such labels may be incorporated into the macromolecular carrier by covalent bonding directly to an atom of the carrier, or the label may be non-covalently or covalently associated with the carrier through a chelating structure or through an auxiliary molecule such as mannitol, gluconate, glucoheptonate, tartrate, and the like.

In general, labeling methodologies vary with the choice of radionuclide and the carrier to be labeled. Labeling methods are described for example in Peters et al. (1986) Lancet 2:946-949; Srivastava et al. (1984) Semin. Nucl. Med 14:68-82; Sinn et al. (1984) J. Nucl. Med. 13:180; McAfee et al. (1976) J. Nucl. Med. 17:480-487; Welch et al., (1977) J. Nucl. Med. 18:558-562; Thakure et al. (1984) Semin. Nucl. Med. 14:107; Danpure et al. (1981) Br. J. Radiol. 54:597-601; Danpure et al. (1982) Br. J. Radiol.

55:247-249; Peters et al. (1982) J. Nucl. Med. 24:39-44; Gunter et al. (1983) Radiology 149:563-566 and Thakur et al. (1985) J. Nucl. Med. 26:518-523.

After the labeling reaction is complete, the reaction mixture may optionally be purified using one or more chromatography steps such as Sep Pack or high performance liquid chromatography (HPLC). Any suitable HPLC system may be used if a purification step is performed, and the yield of cardiovascular imaging agent obtained from the HPLC step may be optimized by varying the parameters of the HPLC system, as is known in the art. Any HPLC parameter may be varied to optimize the yield of the cardiovascular imaging agent of the invention. For example, the pH may be varied, e.g., raised to decrease the elution time of the peak corresponding to the radiolabeled carrier.

Photosensitizer Composition Administration

The photosensitizer compositions of the invention can be administered in a pharmaceutically acceptable excipient, such as water, saline, aqueous dextrose, glycerol, or ethanol. The compositions can also contain other medicinal agents, pharmaceutical agents, carriers, and auxiliary substances such as wetting or emulsifying agents, and pH buffering agents.

Standard texts, such as Remington: The Science and Practice of Pharmacy, 17th edition, Mack Publishing Company, incorporated herein by reference, can be consulted to prepare suitable compositions and formulations for administration, without undue experimentation. Suitable dosages can also be based upon the text and documents cited herein. A determination of the appropriate dosages is within the skill of one in the art given the parameters herein.

A "therapeutically effective amount" is an amount sufficient to effect a beneficial or desired clinical result. A therapeutically effective amount can be administered in one or more doses. In terms of treatment, an effective amount is an amount that is sufficient to palliate, ameliorate, stabilize, reverse or slow the progression of a cardiovascular disease characterized by the presence of vulnerable plaques or otherwise reduce the pathological consequences of the impending rupture. A therapeutically effective amount can be provided in one or a series of administrations.

The effective amount is generally determined by the physician on a case-by-case basis and is within the skill of one in the art.

As a rule, the dosage for *in vivo* therapeutics or diagnostics will vary. Several factors are typically taken into account when determining an appropriate dosage. These factors include age, sex and weight of the patient, the condition being treated, the severity of the condition and the form of the antibody being administered.

The dosage of photosensitizer compositions can range from 0.1 to 10 mg/kg. Methods for administering photosensitizer compositions are known in the art, and are described, for example, in U.S. Patent Nos. 5,952,329, 5,807,881, 5,798,349, 5,776,966, 5,789,433, 5,736,563, 5,484,803 and by (Sperduto et al. (1991) *Int. J. Radiat. Oncol. Biol. Phys.* 21:441-6; Walther et al. (1997) *Urology* 50:199-206). Such dosages may vary, for example, depending on whether multiple administrations are given, tissue type and route of administration, the condition of the individual, the desired objective and other factors known to those of skill in the art. Where the photosensitizer compositions comprises a photoimmunoconjugate, dosages can vary from about 0.01 mg/m² to about 500 mg/m², preferably 0.1 mg/m² to about 200 mg/m², most preferably about 0.1 mg/m² to about 10 mg/m². Ascertaining dosage ranges is well within the skill of one in the art. For instance, the concentration of scFv typically need not be as high as that of native antibodies in order to be therapeutically effective. Administrations can be conducted infrequently, or on a regular weekly basis until a desired, measurable parameter is detected, such as diminution of disease symptoms. Administration can then be diminished, such as to a biweekly or monthly basis, as appropriate.

Compositions of the present invention are administered by a mode appropriate for the form of composition. Available routes of administration include subcutaneous, intramuscular, intraperitoneal, intradermal, oral, intranasal, intrapulmonary (i.e., by aerosol), intravenously, intramuscularly, subcutaneously, intracavity, intrathecally or transdermally, alone or in combination with other pharmaceutical agents. Therapeutic compositions of photosensitizers are often administered by injection or by gradual perfusion.

Compositions for oral, intranasal, or topical administration can be supplied in solid, semi-solid or liquid forms, including tablets, capsules, powders, liquids, and

suspensions. Compositions for injection can be supplied as liquid solutions or suspensions, as emulsions, or as solid forms suitable for dissolution or suspension in liquid prior to injection. For administration via the respiratory tract, a preferred composition is one that provides a solid, powder, or liquid aerosol when used with an appropriate aerosolizer device. Although not required, compositions are preferably supplied in unit dosage form suitable for administration of a precise amount. Also contemplated by this invention are slow release or sustained release forms, whereby a relatively consistent level of the active compound are provided over an extended period.

Another method of administration is intravascular, for instance by direct injection into the blood vessel, plaque or surrounding area.

Further, it may be desirable to administer the compositions locally to the area in need of treatment; this can be achieved, for example, by local infusion during surgery, by injection, by means of a catheter, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as silastic membranes, or fibers. A suitable such membrane is Gliadel® provided by Guilford Pharmaceuticals Inc.

Following administration of the photosensitizer composition, it is necessary to wait for the photosensitizer to reach an effective tissue concentration at the site of the plaque before photoactivation. Duration of the waiting step varies, depending on factors such as route of administration, tumor location, and speed of photosensitizer movement in the body. In addition, where photosensitizer compositions target receptors or receptor binding epitopes, the rate of photosensitizer uptake can vary, depending on the level of receptor expression on the surface of the cells. For example, where there is a high level of receptor expression, the rate of binding and uptake is increased. Determining a useful range of waiting step duration is within ordinary skill in the art and may be optimized by utilizing fluorescence optical imaging techniques.

Devices and Methods For Photosensitizer Composition Activation

Following the waiting step, the photosensitizer composition is activated by photoactivating light applied to the site of the plaque. This is accomplished by applying light of a suitable wavelength and intensity, for an effective length of time, at the site of

the plaque. As used herein, "photoactivation" means a light-induced chemical reaction of a photosensitizer, which produces a biological effect.

Target tissues are illuminated, preferably with red light. Given that red and/or near infrared light best penetrates mammalian tissues, photosensitizers with strong absorbances in the 600 nm to 900 nm range are optimal for PDT. The suitable wavelength, or range of wavelengths, will depend on the particular photosensitizer(s) used. Wavelength specificity for photoactivation depends on the molecular structure of the photosensitizer. Photoactivation occurs with sub-ablative light doses. Determination of suitable wavelength, light intensity, and duration of illumination is within ordinary skill in the art.

For photoactivation, the wavelength of light is matched to the electronic absorption spectrum of the photosensitizer so that photons are absorbed by the photosensitizer and the desired photochemistry can occur. Except where the vessels being treated are very superficial, the range of activating light is typically between 600 and 900 nm. This is because endogenous molecules, in particular hemoglobin, strongly absorb light below 600 nm and therefore capture most of the incoming photons (Parrish et al., (1978) Optical properties of the skin and eyes. New York, NY: Plenum). The net effect would be the impairment of penetration of the activating light through the tissue. The reason for the 900 nm upper limit is that energetics at this wavelength may not be sufficient to produce $^1\text{O}_2$, the activated state of oxygen which, without wishing to necessarily be bound by any one theory, is perhaps critical for successful PDT. In addition, water begins to absorb at wavelengths greater than about 900 nm.

The effective penetration depth, δ_{eff} , of a given wavelength of light is a function of the optical properties of the tissue, such as absorption and scatter. The fluence (light dose) in a tissue is related to the depth, d , as: $e^{-d/\delta_{\text{eff}}}$. Typically, the effective penetration depth is about 2 to 3 mm at 630 nm and increases to 5 to 6 mm at longer wavelengths (700-800 nm) (Svaasand and Ellingsen, (1983) Photochem Photobiol. 38:293-299). These values can be altered by altering the biologic interactions and physical characteristics of the photosensitizer. In general, photosensitizers with longer absorbing wavelengths and higher molar absorption coefficients at these wavelengths are more effective photodynamic agents.

PDT dosage depends on various factors, including the amount of the photosensitizer administered, the wavelength of the photoactivating light, the intensity of the photoactivating light, and the duration of illumination by the photoactivating light. Thus, the dose of PDT can be adjusted to a therapeutically effective dose by adjusting one or more of these factors. Such adjustments are within ordinary skill in the art.

The light for photoactivation can be produced and delivered to the plaque site by any suitable means known in the art. Photoactivating light can be delivered to the plaque site from a light source, such as a laser or optical fiber. Preferably, the photoactivating light is delivered by optical fiber devices that directly illuminate the plaque site. For example, the light can be delivered by optical fibers threaded through small gauge hypodermic needles. Light can be delivered by an appropriate intravascular catheter, such as those described in U.S. Patent Nos. 6,246,901 and 6,096,289, which can contain an optical fiber. Optical fibers can also be passed through arthroscopes. In addition, light can be transmitted by percutaneous instrumentation using optical fibers or cannulated waveguides. For open surgical sites, suitable light sources include broadband conventional light sources, broad arrays of LEDs, and defocused laser beams.

Delivery can be by all methods known in the art, including transillumination. Some photosensitizers can be activated by near infrared light, which penetrates more deeply into biological tissue than other wavelengths. Thus, near infrared light is advantageous for transillumination. Transillumination can be performed using a variety of devices. The devices can utilize laser or non-laser sources, (e.g., lightboxes or convergent light beams).

Where treatment is desired, the dosage of photosensitizer composition, and light activating the photosensitizer composition, is administered in an amount sufficient to produce a phototoxic species. For example, where the photosensitizer composition includes chlorin_{e6}, administration to humans is in a dosage range of 0.5-10 mg/kg, preferably 1-5 mg/kg more preferably 2-4 mg/kg and the light delivery time is spaced in intervals of 30 minutes to 3 days, preferably 12 hours to 48 hours, and more preferably 24 hours. The light dose administered is in the range of 20-500 J/cm, preferably 50-300

J/cm and more preferably 100-200 J/cm. The fluence rate is in the range of 20-500 mw/cm, preferably 50-300 mw/cm and more preferably 100-200 mw/cm. There is a reciprocal relationship between photosensitizer compositions and light dose, thus, determination of suitable wavelength, light intensity, and duration of illumination is within ordinary skill in the art.

Preferably, the phototoxic species induces apoptosis and not necrosis of the cells comprising the vulnerable plaque. Lowering the fluence rate will favor apoptosis (e.g., less than 100 mw/cm, e.g., 10-60 mw/cm, for chlorin_{e6}). Determination of a suitable fluence rate for a photosensitizer composition is within ordinary skill in the art.

Where the fluorescent composition comprises a photoactive dye, the wavelength and power of light can be adjusted according to standard methods known in the art to control the production of phototoxic species. Thus, under certain conditions (e.g., low power, low fluence rate, shorter wavelength of light or some combination thereof), a fluorescent species is produced from the photoactive dye and any reactive species produced has a negligible effect. These conditions are easily adapted to bring about the production of a phototoxic species. For example, where the photoactive dye comprises chlorin_{e6}, the light dose administered to produce a fluorescent species and an insubstantial reactive species is less than about 10 J/cm, preferably less than about 5 J/cm and more preferably less than about 1 J/cm. Determination of suitable wavelength, light intensity, and duration of illumination is within ordinary skill in the art.

In a preferred embodiment, photoactivation can be carried out using by a specially designed intravascular device that delivers excitation light to the plaque surface inside the artery and receives emitted fluorescence or other detectable signals (e.g., heat or radioactivity) that are transmitted to an analysis instrument. The same device can optionally be used to deliver therapeutic light when a fluorescent signal, or other measurable signal (e.g., heat or radioactivity) is detected.

Fig. 1A illustrates a detection/treatment system 100 for detecting and/or targeting and/or treating vulnerable plaque in accordance with an embodiment of the invention. As shown in Fig. 1A, detection/treatment system 100 may include a control unit 105 and a detection/treatment unit 110, which may include a light source/laser 113,

and a detection/treatment device 115, which may include a probe, a catheter, and so forth.

Control unit 105 may include a power supply, for example, control unit may be coupled to a power source, for supplying power to detection/treatment unit 110. Control unit 105 may also include a computing device having control hardware and/or software for controlling, based on inputted parameters and/or detected properties, detection/treatment unit 110, light source/laser 113 and detection/treatment device 115.

Fig. 1B is a diagram illustrating a configuration of control unit 105 in accordance with an embodiment of the invention. As shown in Fig. 1B, control unit 105 may comprise a computing device 125, which may be a general purpose computer (such as a PC), workstation, mainframe computer system, and so forth. Computing device 125 may include a processor device (or central processing unit "CPU") 130, a memory device 135, a storage device 140, a user interface 145, a system bus 150, and a communication interface 155. CPU 130 may be any type of processing device for carrying out instructions, processing data, and so forth. Memory device 135 may be any type of memory device including any one or more of random access memory ("RAM"), read-only memory ("ROM"), Flash memory, Electrically Erasable Programmable Read Only Memory ("EEPROM"), and so forth. Storage device 140 may be any data storage device for reading/writing from/to any removable and/or integrated optical, magnetic, and/or optical-magneto storage medium, and the like (e.g., a hard disk, a compact disc-read-only memory "CD-ROM", CD-ReWritable "CD-RW", Digital Versatile Disc-ROM "DVD-ROM", DVD-RW, and so forth). Storage device 140 may also include a controller/interface (not shown) for connecting to system bus 150. Thus, memory device 135 and storage device 140 are suitable for storing data as well as instructions for programmed processes for execution on CPU 130. User interface 145 may include a touch screen, control panel, keyboard, keypad, display or any other type of interface, which may be connected to system bus 150 through a corresponding input/output device interface/adaptor (not shown). Communication interface 155 may be adapted to communicate with any type of external device, including detection/treatment unit 110. Communication interface 155 may further be adapted to communicate with any system or network (not shown), such as one or more

computing devices on a local area network (“LAN”), wide area network (“WAN”), the internet, and so forth. Interface 155 may be connected directly to system bus 150, or may be connected through a suitable interface (not shown). Control unit 105 may, thus, provide for executing processes, by itself and/or in cooperation with one or more additional devices, that may include algorithms for controlling detection/treatment unit 110 in accordance with the present invention. Control unit 105 may be programmed or instructed to perform these processes according to any communication protocol, or programming language on any platform. Thus, the processes may be embodied in data as well as instructions stored in memory device 135 and/or storage device 140 or received at interface 155 and/or user interface 145 for execution on CPU 130.

Referring back to Fig. 1A, detection/treatment unit 110 may be a handheld device, an automated apparatus, and the like. As shown in Fig. 1A, detection/treatment device 115 may be inserted and extended into a blood vessel 120, such as an artery, in tissue 125. Detection/treatment device 115 may be a handheld device, an automated apparatus, and the like. It is further noted that the elements of detection/treatment system 100 may be integrated into a single physical unit or may comprise any number of discrete units, such that any number of these elements or the functionality thereof, may be incorporated into a physical device. As will be described in further detail below, detection/treatment device 115 may include a number of light delivery elements for delivering detected light from targeted plaque, delivering therapeutic light, and/or delivering detection/excitation light.

In accordance with an embodiment of the invention, light source 113 may include a pulse blue laser for delivering detection or excitation light via detection/treatment device 115. Depending on the dye and/or excitation effect on target plaque as described above, reflected and/or emitted light from the target plaque may include light with a particular wavelength and/or frequency, which may then be detected through detection/treatment device 115. A large number of fluorescent probes (e.g., photosensitizers, fluorescent dyes or photoactive dyes) and methods of use thereof (e.g., excitation and emission wavelengths), are described in the Molecular Probes, Inc. catalog, (Handbook of Fluorescent Probes and Research Chemicals, 6th Edition by Richard Haugland), the contents of which are hereby incorporated by reference.

In accordance with an embodiment of the invention where in the fluorescent composition or photosensitizer composition includes chlorin_{e6}, detection/excitation light may include a wavelength of 337 nm (for example, nitrogen laser), therapeutic light may include a wavelength of 405 nm (for example, pump dye laser), and light or fluorescence emitted from target plaque as a result of excitation by detection/excitation light may include a wavelength of 666-668 nm. The power of detection/excitation light may, for example, be adjusted in accordance with the specific excitation or emission wavelength of the particular fluorescent or photosensitizer composition used. The power of detection/excitation light may, for example, be adjusted in accordance with a size and/or dimension of blood vessel 120. The power of therapeutic light may, for example, be adjusted in accordance with a size and/or dimension of blood vessel 120, and/or the level of light detected from target plaque.

In accordance with an embodiment of the invention, detection/treatment system 100 may include a number of configurations and instruments. Algorithms that are designed for different types of procedures, configurations and/or instruments may be included for control unit 105.

It is noted that detection/treatment system 100 may be controlled remotely. For example, the link between control unit 105 and detection/treatment unit 110 may be a remote link (wired or wireless) providing control unit 105 remote control over light source 113 and detection/treatment device 115.

While the above exemplary detection/treatment system 100 is illustrative of the basic components of a system suitable for use with the present invention, the architecture shown should not be considered limiting since many variations of the hardware configuration are possible without departing from the present invention.

The present invention is additionally described by way of the following illustrative, non-limiting Examples, that provide a better understanding of the present invention and of its many advantages.

As described before, target plaque may accumulate on the wall of blood vessels, e.g. arteries, and the like. Thus, detection/treatment device 115 embodying the present invention may include a probe/catheter and the like, as described below, which may include a number of elements for detecting the target plaque on the wall of these blood

vessels, distinguishing the target plaque from non-target plaque and/or treating the target plaque without obstructing the blood flow through these vessels.

Figs. 2A, 2B, 2C, 2D, 2E and 2F are diagrams showing a probe/catheter 200 in accordance with an embodiment with the present invention. As shown in Fig. 2A, probe/catheter 200 may include an external unit 202 and an extendible internal unit, which may include a number of light delivery element(s) 205 and light deflection element(s) 210 and a tip 215. As an example, external unit 202 may include any plastic and/or metallic material (e.g., nitinol alloy) and the like. Fig. 2A illustrates probe/catheter 200 with its internal unit retracted within and extended from external unit 202, and Fig. 2B illustrates probe/catheter 200 with its internal unit extended and deployed. In accordance with an embodiment of the invention, the internal unit may be extended and deployed to detect target plaque, then retracted to move probe/catheter 200 to a different area within, say, blood vessel 120. For example, probe/catheter 200 may be used to scan blood vessel 120 where probe/catheter 200 is moved along blood vessel 120 and the internal unit is extended every one to six millimeters to make a detection. A guidewire 223 may be used to guide probe/catheter 200 along blood vessel 120 and/or extend/retract the internal unit (e.g., light delivery element(s) 205 and light deflection elements 210, and so forth) from/into external unit 202. As an example, guidewire 223 may include any plastic and/or metallic material (e.g., nitinol alloy) and the like. Light deflection element(s) 210 may include a smooth surface for contacting the wall of blood vessel 120, thus allowing detection while probe/catheter 200 is being moved. Detection may be made without contacting the wall or probe/catheter 200 may also be stopped to make such a detection. Probe/catheter 200 may include four light delivery elements 205, each including a light deflection element 210. Each of the four light delivery elements 205 may be disposed such that the corresponding light deflection elements 210 form a circumference separated by 90 degrees, as shown by the cross-sectional views in Figs. 2C and 2D. It is noted that probe/catheter 200 may include any number of light delivery element(s) 205 (and light deflection element(s) 210) separated by a corresponding angle around a circumference for covering a divided area of the surrounding wall of blood vessel 120. Probe/catheter 200 may also be rotatable to cover the circumference of blood vessel 120. In accordance with a

preferred embodiment of the invention, probe/catheter 200 may include three to six light delivery elements 205 (and light deflection elements 210). It is noted, of course, that light delivery elements 205 may be split from a single element connected to detection/treatment unit 110 or they may be separately connected to detection/treatment unit 110.

As will be described in further detail below, light deflection element(s) 210 may deflect external light received from blood vessel 120 into light delivery element(s) 205, which may then deliver the received light to detection/treatment unit 110 and/or control unit 105 for analysis. Light deflection element(s) 210 may also deflect detection/excitation light, which may be delivered from detection/treatment unit 110 through light delivery element(s) 205, and shine the detection/excitation light onto a target area in blood vessel 120. And so, reflected light and/or light emitted from excited target plaque may be received as described above. Depending on the dye and/or excitation effect on target plaque as described before, the target plaque may reflect and/or emit light having a particular wavelength and/or frequency. Thus, target plaque may be identified and located by detecting and identifying light having such a particular wavelength and/or frequency from the light received from blood vessel 120.

Light delivery element(s) 205 may include an optical fiber for delivering light received at its corresponding light deflection element(s) 210 to treatment unit 110 and/or control unit 105. Light delivery element(s) 205 may also deliver detection/excitation light from light source 113 to its corresponding light deflection element(s) 210 where it is deflected and shone onto blood vessel 120. As shown in Fig. 2A, light delivery element(s) 205 may extend to and joined at a tip 215.

As shown in Fig. 2B, light delivery element(s) 205 may move outward so that light deflection element(s) 210 are moved towards the surrounding wall of blood vessel 120, thus allowing better plaque detection. In accordance with an embodiment of the invention, light delivery element(s) 205 may include a rigid and/or spring-like structure, for example, a plastic structure, such that the structure expands when extended, as shown in Fig. 2B, and may be compressed within external unit 202 when retracted, as shown in Fig. 2A. The rigid structure may include any elastic material so that the

structure expands to substantially the same size and shape every time it is extended as shown in Fig. 2B.

In accordance with an embodiment of the invention, probe/catheter 200 may include a vessel (or “balloon”) 220 that may be expanded by filling it with a fluid. Thus, when extended as shown in Fig. 2B, vessel 220 may be filled with fluid and expanded, pushing light deflection element(s) 210 towards the surrounding wall of blood vessel 120. The fluid may be any non-toxic fluid, such as saline and so forth. As an example, vessel 220 may include any elastic material, such as rubber or latex, and the like. Control unit 105 and/or detection/treatment unit 110 may control fluid flow to and from vessel 220 so that fluid is delivered thereto when probe/catheter 200 is extended, and drained when probe/catheter 200 is retracted. Advantageously, the amount of fluid may be controlled so as to fit the size of the surrounding blood vessel 120. In other words, less fluid may be delivered if blood vessel 120 is relatively small and more fluid may be delivered if blood vessel 120 is relatively large. Thus, light deflection element(s) 210 may be moved towards the wall of a blood vessel 120 of any size, while preventing light deflection element(s) 210 from being pressed against the wall of a smaller blood vessel 120.

Figs. 2C and 2D are diagrams showing cross-sectional views of Figs. 2A and 2B, respectively. When expanding vessel 220 or otherwise moving light deflection element(s) 210 towards the wall of blood vessel 120, it is important that blood flow through blood vessel 120 be unhindered. Therefore, in accordance with an embodiment of the invention, vessel 220 may include a number of rigid element(s) 225 so that only a particular portion of vessel 220 expands when filled with fluid. As an example, rigid element(s) 225 may include a rigid material, for instance any plastic and/or metallic material (e.g., nitinol alloy), and the like. As shown in Figs. 2C and 2D, vessel 220 may include four rigid element(s) 225, such as plastic ribbings, and the like. As shown in Fig. 2D, rigid element(s) 225 may hold vessel 220 in place where only regions of vessel 220 that are adjacent light delivery element(s) 205 and light deflection element(s) 210 may expand outward. Therefore, vessel 220 does not substantially block blood vessel 120 when it is expanded. Light deflection element(s) 210 may, thus, be moved outward to the wall of blood vessel 120 without obstructing blood flow.

Figs. 2E and 2F illustrate cross-sectional views of probe/catheter 200 in accordance with an embodiment of the invention. As shown in Figs. 2E and 2F, vessel 220 may include an isolated chamber corresponding to a particular light deflection element 210. Therefore, each of any number of particular light deflection element(s) 210 may correspond to such a chamber in vessel 220 so that element(s) 210 can be individually moved towards and away from the wall of blood vessel 120, by individually inflating and deflating each chamber. For example, as shown in Fig. 2F, a chamber 230 may be individually deflated (i.e., drained of fluid), in the event that therapeutic light may be directed to the corresponding region on blood vessel 120, say, from tip 215, in the event that the corresponding region need not be detected or monitored for any reason, or to fit to a particular dimension of a blood vessel.

Figs. 3A, 3B and 3C are diagrams illustrating a probe/catheter 300 in accordance with an embodiment of the invention. Probe/catheter 300 as shown in Figs. 3A and 3B is similar to probe/catheter 200 shown in Figs. 2A and 2B, respectively, except that probe/catheter 300 may include only one light delivery element 205 and corresponding light deflection element 210. Advantageously, the cross-sectional area of probe/catheter 300, when extended and deployed, may be further reduced. For example, as shown in Fig. 3C, probe/catheter 300 may include only one prong compared to the four prongs shown in Fig. 2D for probe/catheter 200. As a result, blood flow obstruction may be further reduced. Probe/catheter 200 may include a platform 305 for supporting, say, vessel 220. As an example, platform 305 may include a rigid material, for instance any plastic and/or metallic material (e.g., nitinol alloy), and the like, so that it is held in place while vessel 220 expands and pushes light deflection element 210 outward. As mentioned before, light delivery element 205 may include a rigid structure that pushes outward when extended from external unit 202. Platform 305 may support such a structure.

Figs. 4A and 4B show a probe/catheter 400 in accordance with an embodiment of the invention. As shown in Figs. 4A and 4B, probe/catheter 400 may include light delivery elements 205 disposed on a rigid structure that is compressed when enclosed in external unit 202, as shown in Fig. 4A, and expands when extended, as shown in Fig. 4B. As described before, the rigid structure may include any elastic material so that the

structure expands to substantially the same size and shape every time it is extended as shown in Fig. 4B. As an example, the rigid structure may include any plastic and/or metallic material (e.g., nitinol alloy) and the like.

Figs. 5A and 5B are diagrams illustrating light delivery element 205 and light deflection element 210 in accordance with respective embodiments of the invention. As shown in Fig. 5A, light deflection element 210 may include a reflective surface 505 and/or a refractive element 510 for deflecting light from a target area back to detection/treatment unit 110 through light delivery element 205, and/or deflecting detection/excitation light from light source 113 to the target area. In accordance with an embodiment of the invention, light source 113 may include a light source for therapeutic light having a difference wavelength and/or frequency. Thus, light deflection element 210 may deflect only detection/excitation light, while allowing therapeutic light to pass through. Referring back to Figs. 2A and 2B, the passed through therapeutic light may be deflected out at tip 215 for effecting treatment on the surrounding wall of blood vessel 120. Probe/catheter 200 may further be extended and/or retracted partially when effecting treatment so as to ensure that therapeutic light from tip 215 reaches the areas covered by light deflection element(s) 210.

Figs. 5B illustrates light deflection element 210 that may be used in probe/catheter 400, as shown in Figs. 4A and 4B, in accordance an embodiment of the invention. As shown in Fig. 5B, a therapeutic light deflection unit 515 may be placed adjacent light deflection element 210. Since it is advantageous to target therapeutic light more broadly to cover tissue surrounding the detected plaque, therapeutic light deflection unit 515 may include a refractive material for spreading or diffusing the therapeutic light in all directions to cover the surrounding wall of blood vessel 120. In accordance with an embodiment of the invention, therapeutic light deflection unit 515 may also include a reflective element 520 for targeting the therapeutic light to a general direction or a particular area. Thus, referring back to Figs. 4A and 4B, a therapeutic light deflection unit 515 may be disposed at the end, or tip, of each light deflection element 210.

In accordance with an embodiment of the invention, detection/excitation light and therapeutic light may be carried on separate light delivery elements.

Figs. 6A, 6B, and 6C illustrate a probe/catheter 600 in accordance with an embodiment of the present invention. As shown in Fig. 6A, probe/catheter 600 may include a detector 605, such as a scintillation detector, and the like, for detecting emitted and/or reflected light, radioactive signals (e.g., gamma rays, beta rays, and so forth), nuclear isotopes, radio frequency/microwave signals, magnetic fields, electric fields, temperature (e.g., heat), vibration, and so forth. By detecting any one or more of the foregoing, target plaque may be identified and/or located from surrounding plaque/tissue. As further shown in Fig. 6A, probe/catheter 600 may also include a therapeutic light deflector 610, such as a diffusing fiber, and the like, for diffusing therapeutic light to surrounding plaque/tissue. As shown in Fig. 6B, detector 605 may be independently retracted so that therapeutic light may be directed to the general direction or particular area where target plaque/tissue is detected. Furthermore, as shown in Fig. 6C, therapeutic light deflector 610 may include a reflective element 615, such as a shield, and the like, to block therapeutic light from diffusing to a non-target direction. For example, after detector 605 detects target plaque/tissue, it may be retracted and therapeutic light deflector 610 and reflective element 615 may diffuse therapeutic light only to the general direction and/or target area covered by detector 605. In accordance with an embodiment of the invention, probe/catheter 600 may be rotatable in, say, blood vessel 120 so that detector 605 and therapeutic light may be directed in any direction therewithin.

The present invention is additionally described by way of the following illustrative, non-limiting Examples, that provide a better understanding of the present invention and of its many advantages.

EXAMPLES

Example 1

Preparation and purification of photosensitizer compositions

A photosensitizer composition comprising chlorin_{e6} ("c_{e6}") coupled to maleylated-albumin) was prepared for optimal targeting to macrophages of a vulnerable plaque animal model system.

Results

Four photosensitizer compositions were studied (i.e., two BSA- c_{66} conjugates and their maleylated counterparts). The N-hydroxy succinimide (NHS) ester of c_{66} was prepared by reacting approximately 1.5 equivalents of dicyclohexylcarbodiimide and approximately 1.5 equivalents of NHS with approximately 1 equivalent of c_{66} (Porphyrin Products, Logan, UT) in dry DMSO. After standing in the dark at room temperature for approximately 24 hours, the NHS ester was frozen in aliquots for further use. BSA (Sigma Chemical Co, St Louis, MO) (approximately 2 X 50 mg) was dissolved in NaHCO_3 buffer (0.1 M, pH 9.3, approximately 3 ml), and approximately 30 μl and approximately 120 μl of c_{66} -NHS ester added to respective tubes with vortex mixing. After standing in the dark at room temperature for approximately 6 hours, the crude conjugate preparations were each divided into two approximately equal parts. One portion of each of the conjugate preparations was maleylated by adding solid maleic anhydride (approximately 20 mg) to the protein preparation in portions and with vortex mixing, and by adding saturated NaHCO_3 solution as needed to keep the pH above approximately 7.0 (Takata et al. (1989) *Biochim. Biophys. Acta* 984:273). The reaction mixture was allowed to stand at room temp in the dark for approximately 3 hours (Figure 7). Unmodified BSA was also maleylated to act as a control and as a competitor for the cellular uptake of conjugates.

Crude conjugate preparations (approximately 5 mg/ml) were added to approximately 10X volume of acetone (ACS grade) slowly at approximately 4°C, and were kept at approximately 4°C for approximately 6 hours, followed by centrifugation at about 4000 X g for approximately 15 minutes at about 4°C. The supernatant was removed and the pellet again suspended in approximately the same volume of acetone and the centrifugation repeated. After each precipitation step the preparation was monitored by thin layer chromatography (TLC). Approximately five precipitation steps were necessary to completely remove non-covalently bound chlorin species. Finally, the pellet was dissolved in approximately 2 ml PBS and dialyzed approximately twice against 20 L PBS overnight to remove traces of acetone.

Sephadex G50 column chromatography was carried out by applying the reaction mixture from conjugation of approximately 50 mg BSA with approximately 5 mg c_{66} -NHS ester to a 50 x 1 cm Sephadex column that was eluted with PBS at about 4°C. The absorbance of the eluted fractions was monitored at 400 nm and at 280 nm.

A problem that can be encountered in the preparation of covalent conjugates of tetrapyrrole photosensitizer (PS) with proteins is the tendency of the dye to form tightly bound non-covalent complexes, as well as conjugates. These mixtures can be difficult to separate into pure conjugate and non-bound dye. This is illustrated by the attempted use of a Sephadex G50 column to separate the BSA- c_{66} conjugate from unreacted c_{66} -NHS ester and its subsequent reaction products. Monitoring of the eluted fractions at 400 nm and at 280 nm showed a single peak that contained both c_{66} and protein. However, when the material obtained from combining the fractions was examined by TLC, as shown in Figure 8A, it was apparent that there was a considerable amount of unbound dye present. Lane 1 on the TLC shows the single peak isolated from the size exclusion column and demonstrates that there was still considerable unbound c_{66} present as a fast running spot. When this material was used in cell-uptake experiments, it was difficult to distinguish receptor targeting between J774 and EMT-6 cell due to indiscriminate uptake of unbound c_{66} by both receptor-positive and receptor-negative cells. Likewise, lane 3 shows the crude mixture after maleylation and that there was unbound c_{66} present.

Therefore, the conjugates were purified using an acetone precipitation that allowed the lipophilic c_{66} species to be retained in the acetone supernatant and the precipitated conjugates to be redissolved in a purified form. The sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gels were viewed by fluorescence imaging to localize the c_{66} after staining with Coomassie Blue. Figure 8B shows the corresponding fluorescence and Coomassie images of BSA, BSA mixed with free c_{66} and conjugates (BSA- c_{66} 1 and mal-BSA- c_{66} 1) after Sephadex column chromatography, but before acetone precipitation. The mixture of BSA and c_{66} (lanes 2a and 2b) showed that no fluorescence is retained by the protein band on the gel, thus demonstrating that a fluorescent band localizing with the protein is evidence of covalent conjugation. The

lanes of the conjugates (3a and 3b, 4a and 4b) show that a fluorescent band running at the gel front remained after Sephadex chromatography.

The efficiency of the purification by acetone precipitation of the conjugates was confirmed by the gel electrophoresis images shown in Figure 8C. It can be seen that the fast running fluorescent band disappeared from both the BSA- c_{66} and the mal-BSA- c_{66} (lanes 2c and 2d, 3c and 3d), while the TLC also showed the disappearance of the fast running spot (Figure 8A, lanes 2 and 4)

The concentrations of the constituents in the conjugates and, hence the substitution ratios, were measured by absorbance spectroscopy. An aliquot of the conjugate was diluted in approximately 0.1 M NaOH/1% SDS and absorbance between 240 nm and 700 nm scanned. The extinction coefficient of BSA at 280 nm is approximately $47000 \text{ cm}^{-1}\text{M}^{-1}$ (Markwell et al. (1978) Anal Biochem 87:206) while the extinction coefficient of c_{66} at 400 nm is approximately $150000 \text{ cm}^{-1}\text{M}^{-1}$. Thin layer chromatography was performed on silica gel plates (Polygram SIL G/UV254, Macherey Nagel, Duren, Germany). The chromatograms were developed with an approximately 1:1 mixture of approximately 10% aqueous ammonium chloride and methanol, and spots were observed with fluorescence and absorbance imaging. SDS-PAGE was carried out essentially according to the methods known in the art (Laemmli (1970) Nature 227:680). Gradients of 4-10% acrylamide were used in a non-reducing gel and c_{66} was localized on the gel by a fluorometer (excitation at 400-440 nm bandpass filter, emission scanned from 580-720 nm longpass filter (ChemiImager 4000, Alpha Innotech Corp, San Leandro, CA). Proteins were localized by Coomassie blue staining.

The UV-visible absorption spectra of the purified mal-BSA- c_{66} conjugates with the two substitution ratios measured at approximately equal protein concentrations are shown in Figure 9, together with free c_{66} at approximately the same concentration as was present in mal-BSA- c_{66} 2. Similar spectra were obtained for BSA- c_{66} 1 and 2. Using the values for molar extinction coefficients of BSA at 280 nm of approximately $47000 \text{ cm}^{-1}\text{M}^{-1}$ (Markwell et al (1978) Anal Biochem 87:206) and c_{66} at 400 nm of approximately $150000 \text{ cm}^{-1}\text{M}^{-1}$, and correcting for the small absorbance of c_{66} at 280

nm, then the substitution ratios can be calculated to be mal-BSA- c_{66} 1 ratio equals approximately 1 protein to approximately 1 dye, and mal-BSA- c_{66} 2, ratio equals approximately 1 protein to approximately 3 dye.

Example 2

Macrophage-Targeting of Photosensitizers

The photosensitizer composition comprising chlorin c_{66} coupled to maleylated-albumin described in Example 1 was shown to accumulate in the macrophage-rich plaques of an animal model system that are analogous to vulnerable plaques in humans. Thus, methods of the present invention provide highly specific intravascular detection and therapy of vulnerable plaques.

Cell Culture

J774.A1 (J774) and RAW 264.7 mouse macrophage-like cell lines, together with EMT-6 mouse mammary fibrosarcoma cells, were obtained from ATCC (Rockville, MD). Cells were grown in RPMI 1640 media containing HEPES, glutamine, 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 μ g/ml streptomycin. They were passaged by washing with phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+} and by adding trypsin-EDTA to the plate for 10 minutes at 37°C.

Rabbits

Male New Zealand white rabbits weight 2.5-3.0 kg (Charles River Breeding Lab) were maintained on a 2% cholesterol-6% peanut oil diet (ICN) for 6 weeks.

Results

For cellular uptake studies, cells were grown to approximately 90% confluency in twenty-four well plates and the conjugate or photosensitizer was added in about 1 ml medium containing approximately 10% serum to each well. The concentration range for the conjugates and free c_{66} was between approximately 0.5 and 4 μ M c_{66} equivalent and the incubation time was approximately 3 hours. After incubation at 37°C, the medium was removed and cells were washed about three times with approximately 1 ml sterile PBS and incubated with approximately 1 ml trypsin-EDTA for about 20 minutes (OVCAR-5) or 60 minutes (J774). The cell suspension was then removed and

centrifuged (about 5 minutes at approximately 250 X g). The trypsin supernatant was aspirated and retained and the pellets (frequently visibly fluorescent under long wave UV) were dissolved in about 1.5 ml of approximately 0.1M NaOH / 1% SDS for at least about 24 hours to give a homogenous solution. The trypsin supernatant was checked for the presence of fluorescence to quantify any surface binding which might easily be removed by trypsin. The fluorescence was measured using an excitation wavelength of 400 nm and the emission scanned from 580 to 700 nm in order to calculate the peak area ($\lambda_{\text{max}} = 664 \text{ nm}$). A series of dilutions in approximately 1.5 ml 0.1M NaOH / 1% SDS of known concentrations of each separate conjugate and photosensitizer was scanned for fluorescence as above in order to prepare calibration curves to allow for quantitation of the c_{e6} by conversion of the measured peak areas into mol c_{e6} equivalent. The protein content of the entire cell extract was then determined by a modified Lowry method (Marwell et al (1978) Anal Biochem 87:296) using BSA dissolved in approximately 0.1M NaOH/1% SDS to construct calibration curves. Results were expressed as mol of c_{e6} per mg cell protein. For measuring the cellular uptake at 4°C, pre-cooled growth media was used and the plates with cells were cooled to about 4°C in an ice-bath for approximately 20 minutes before the addition of photosensitizer solutions as well as after the addition. The incubation was carried out in the normal atmosphere in the dark (e.g., plates wrapped in aluminum foil).

Cells were seeded in 24 well plates, at densities of approximately 100,000 cells in about 1 ml medium. After about 24 hours, the cells were given about 1 ml fresh medium containing 10% serum and a specific conjugate or free c_{e6} (c_{e6} equivalent concentration of approximately 4 nmoles per well) and incubated for about 3 hours at 37°C. Immediately prior to illumination, the cells were washed about 3 times with PBS with $\text{Mg}^{2+}/\text{Ca}^{2+}$ and the wells were replenished with approximately 1 ml medium containing HEPES and about 10% FCS. Light (660 nm) was delivered from beneath the wells from a diode laser at a fluence rate of about 50 mW/cm² via a fiber optic coupled microscope objective. Wells were illuminated in blocks of four defined by a black mask placed beneath the 24 well plate. Fluences were about 2, 5, and 10 J/cm². After completion of illumination, the dishes were returned to the incubator for a further

approximately 24 hour incubation. Cell survival was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which measures mitochondrial dehydrogenase activity. It has been extensively used for measuring viability of cell cultures after PDT and has been shown to have close correlation with colony forming assays (McHale et al (1988) Cancer Letters 41:315). Approximately twenty-four hours post illumination, the cells were given fresh media and about 100 μ l MTT (5 mg/ml) solution was added to each well and cells were incubated at 37°C. After approximately 1 hour incubation, the supernatant medium was gently aspirated and about 1 ml of DMSO was added to lyse the cells and dissolve the deep blue formazan. Plates were gently shaken on an orbital shaker in the dark for approximately 15 min to complete the dissolution of any formazan crystals and the blue DMSO solution was transferred to 96 well plates (about 200 μ l per well, 5 wells per well of 24-well plate). Absorbance was read on an automated plate reader (Model 2550 EIA, Bio-Rad Laboratories, Hercules, CA) at 570 nm. Data points were the average of 3 wells of the 24 well plate (15 wells of 96 well plate).

The role of scavenger receptors in the uptake of these conjugates was tested by measuring the reduction in the cellular content of photosensitizer produced by competing the uptake with a ligand known to be recognized by the scavenger receptor. The reduction in cellular uptake was then related to protection of the cells from phototoxicity. Increasing amounts of unlabeled mal-BSA were added simultaneously with the conjugates to J774 and OVCAR-5 cells and incubated for about 3 hours. Approximately 0, 50, 100, and 200 μ g/ml mal-BSA were used, representing a range of about 0.25 to 3 fold molar excess of the BSA contained in approximately 4 μ M BSA- c_{66} or mal-BSA- c_{66} . The cellular uptakes and phototoxicities were measured as described above.

Mouse-macrophage cells (J774 or RAW264.7) took up more than ten times as much dye as non-target EMT-6 cells and, upon illumination with modest levels of red light, were killed approximately 1000 times as much. The maleylated conjugates had greater macrophage selectivity and, therefore, higher phototoxicity than their non-maleylated counterparts (Figure 10).

After 1 week on the peanut oil diet, the abdominal aorta was denuded of endothelium by a modified Baumgartener technique. Briefly, each animal was anesthetized with a mixture of ketamine and xylazine and the right femoral artery was isolated. Subsequently, a 4F Fogarty embolectomy catheter was introduced via arteriotomy and advanced under fluoroscopic guidance to the level of the diaphragm. The balloon was then inflated to 3 psi above balloon inflation pressure and three passes were made down the abdominal aorta with the inflated catheter. The femoral artery was subsequently ligated and the wound closed.

For fluorescence localization within *ex vivo* aortas, aortic segments were cut open and flattened and the luminal side examined by spectrofluorometry using either a fiber-bundle based double monochromator spectrofluorimeter (Skin Scan, Spex Figure), where emission spectra (excitation 400 nm, emission 580-720 nm) was collected about every 3 mm across the entire area of the exposed intimal surface, or an optical multichannel analyzer (Figure 11).

For confocal fluorescence microscopy, selected parts of the aortas were snap frozen in liquid nitrogen and approximately 10 – 20 μm frozen sections were prepared. These sections underwent laser scanning confocal fluorescence microscopy to detect the tissue distribution of the C_{60} . The red intracellular fluorescence from C_{60} together with green tissue auto-fluorescence was imaged in the cells in 10 μm frozen sections. Sections were examined with a laser scanning confocal fluorescence microscope. A Leica DMR confocal laser fluorescence microscope (Leica Mikroskopie und Systeme GmbH, Wetzlar, Germany) (excitation 488 nm argon laser) and 4X-40X air immersion lens or a 100X oil immersion objective was used to image at a resolution of 1024 x 1024 pixels. Two channels collected fluorescence signals in either the green range (580 nm dichroic mirror plus 530 nm (+/- 10 nm) bandpass filter) or the red range (580 nm dichroic mirror plus 590 nm longpass filter) and were displayed as false color images. These channels were overlaid using TCS NT software (Version 1.6.551, Leica Lasertechnik, Heidelberg, Germany) to allow visualization of overlap of red and green fluorescence. These sections were also stained by immunohistochemistry using macrophage specific monoclonal antibodies and conventional H&E staining. Other parts of normal and atherosclerotic aorta were cut into small pieces, weighed and

dissolved in sodium hydroxide/SDS and the tissue content of c_{e6} was determined by spectrofluorimetry as previously described (Hamblin et al (2000) Br. J. Cancer 83:1544).

Figure 12 shows an analysis of aortic sections from rabbits injected with or without conjugate (approximately 2 mg/kg in PBS) about 24 hours after injection of the conjugate. Row 1 shows confocal fluorescence micrographs of frozen aortic sections (Red = chlorin e6, Green = elastic lamina auto-fluorescence). Row 2 shows fluorescence emission spectra (excitation = 400 nm) of intimal surface of aortic segments ex vivo. Row 3 shows Hematoxylin and eosin staining of formalin fixed paraffin embedded aortic segments. Row 4 shows Verhoeff's elastic tissue stain. The confocal micrographs showed red fluorescence from the PS (c_{e6}) and green auto-fluorescence principally from the elastic lamina of the arteries. Column 1 shows an atherosclerotic rabbit with no injection of conjugate. There was no red c_{e6} fluorescence in the tissue section, nor any fluorescence signal from the intimal surface. Column 2 shows a normal non-atherosclerotic rabbit injected with conjugate. There is a small amount of red fluorescence visible in the adventitia rather than the intima in the fluorescence micrographs, and a small fluorescence emission signal from the intimal surface. Column 3 shows an atherosclerotic rabbit injected with conjugate. There was a large amount of red fluorescence visible in the plaque and this gave a corresponding large fluorescence emission signal from the intimal surface.

The intimal fluorescence signal was measured from different sections of aortas from atherosclerotic and normal rabbits. The areas of the abdominal aorta that received balloon injury developed greater amounts of plaque than the neighboring thoracic and lower abdominal aortas. The results from the intimal fluorescence measurements were confirmed by extracting sections of the aortas and measuring fluorescence with a spectrofluorimeter that gives a measure of the number of c_{e6} molecules in the tissue sections.

Figure 13 shows a significant fluorescent signal from the intimal surface (determined by Skin Scan) in all sections from atherosclerotic rabbits compared to the corresponding sections of aorta from normal rabbits injected with conjugate, but particularly higher in the sections from the balloon-injured areas. The section 1 depicts

thoracic aorta, section 2 depicts upper abdominal aorta below the diaphragm, section 3 depicts mid-abdominal aorta, section 4 depicts lower abdominal aorta and section 5 depicts pelvic aorta just above bifurcation. At least 6 separate measurements were taken from each artery segment. By the nature of the balloon injury, sections 3 and 4 generally sustained a more severe endothelial injury than other sections and hence developed more severe atherosclerosis. These plaques are extremely rich in macrophages and therefore, are most analogous to vulnerable plaques in humans. Such lesions represent the animal model system used by those of skill in the art to study the features of vulnerable plaques. The signal from atherosclerotic rabbit section 3 was greater than normal control section 3 ($p < 0.0005$) and the signal from atherosclerotic section 4 was greater than normal control section 4 ($p < 0.005$).

The second measurement of intimal surface fluorescence was made by the OMA-LIF system described above. At least 15 separate fluorescence measurements were taken from each artery segment. In addition, the iliac artery through which the balloon was passed also sustained an injury due to its relatively small diameter compared to aortic section 5 and, therefore, developed atherosclerosis compared to the uninjured iliac artery. Figure 13 shows a similar pattern to the Skin Scan measurements that can be seen with highly significant increases in fluorescence in the arteries with inflamed plaque (i.e., balloon injured aorta and iliac). Sections 3, 4 and injured iliac of atherosclerotic compared to normal control had p values < 0.0001 , while section 5 and uninjured iliacs had p values < 0.0005 . Accordingly, the less severe plaques of section 5 are distinguishable from the macrophage-rich plaques of sections 3 and 4. Sections 1 and 2 were not significantly different in atherosclerotic and normal rabbits.

To corroborate the selectivity of the macrophage targeted conjugate for inflamed plaque, the dye molecules were extracted out of the pre-weighed tissue sections by dissolving the tissue in a solvent (1M NaOH/0.2% SDS) designed to preserve c_{66} fluorescence. These dissolved tissue sections were then measured on the spectrofluorimeter and the fluorescent signal was divided by the tissue weight to give a value per gram tissue. At least four pieces of tissue were dissolved for each data point. The differences between atherosclerotic and normal rabbits were significant ($p < 0.05$) for sections 1, 2, and 4. The lower level of significance in this assay was probably due

to the inability to sample as many points as was possible with the surface fluorescence measurement. In addition, it is possible that surface measurement of fluorescence was more sensitive than bulk extraction for detecting macrophage population because macrophages are more likely to be concentrated in the inflamed surface of the plaque.

In Figure 14a, a marked contrast was seen between a large aortic plaque and an area of the abdominal aorta 5 mm beneath the plaque. In Figure 14b, another marked contrast was seen between the balloon injured iliac artery and the contralateral normal artery in the same rabbit. Similarly, Figure 14c shows a contrast between the plaque-laden aorta of an atherosclerotic rabbit and the same area of the aorta in a normal rabbit. These spectra were obtained in a rabbit that had received an overdose of anesthesia. The rabbit received a laparotomy that exposed the abdominal aorta and iliac arteries. The rabbit also had an arteriotomy in the right leg to expose the femoral artery. The fiber-optic catheter of the OMA-LIF apparatus was advanced through the femoral and iliac arteries, to the abdominal aorta, up to the thoracic aorta. Spectra were obtained and the fiber optic catheter pulled back about 5 mm each time successive spectra were obtained. By palpation of the outside of the artery, the position of the catheter in relation to plaques was determined.

Thus, a novel method has been developed for targeting a photosensitizer composition to the activated macrophages of a vulnerable plaque with high specificity.

Example 3

In vivo Photodynamic Therapy

An intravascular fluorescence catheter that efficiently localized a fluorescence signal from a vulnerable plaque in the rabbit coronary (although not limited to rabbit) through flowing blood was developed. In addition, a therapeutic intravascular light delivery system was developed that illuminated the vulnerable plaques through flowing blood with the appropriate wavelength, fluence and fluence rate of light, achieving the desired therapeutic effect.

Results

PDT in rabbit aorta was demonstrated to be possible *in vivo* in living rabbits through flowing blood without undue harm to the rabbits and with no short-term toxicity. The same parameters were used as above (photosensitizer composition, dose

and time interval) in order to be able to correlate treatment effects with previously determined dye localization in plaque lesions. Animals (one atherosclerotic and one normal rabbit, each injected with Mal-BSA-ce6 24 hours previously; and one atherosclerotic rabbit that received no injection) were anesthetized as before and a cylindrical diffusing tipped fiber optic (length of tip = 2 cm, diameter = 1 mm) was advanced to a position midway along the balloon-injured abdominal aorta. The fiber had a SMA connector at the proximal end that can be connected to a diode laser emitting light at approximately 665 nm for Mal-BSA-ce6. Light was delivered at a fluence rate of approximately 100 mW/cm of diffusing tip and a total fluence of approximately 100 J/cm was delivered. At the conclusion of the illumination, the fiber was withdrawn and the arteriotomy and overlying wound were closed. Animals were sacrificed 48 hours later. They received a laparotomy and surgical exposure of the aorta and surrounding tissues (Figure 15A). The top panel of Figure 15A shows light delivery into the abdominal aorta via a diffusing tip catheter inserted into the femoral artery, demonstrating the feasibility of intra-arterial illumination. The middle panel of Figure 15A shows atherosclerotic aorta that is thick such that light did not penetrate to extra-aortic tissue. The bottom panel of Figure 15A shows normal aorta that is thin such that light penetrates to give a slight but definite damage to psoas muscle. Complete aortas and iliac arteries were removed from the PDT treated normal and atherosclerotic rabbits and control (no Mal-BSA-ce6 injection) atherosclerotic rabbit and were examined by histology using H&E, Masson Trichrome and Verhoeff's stain.

The two rabbits that received both the photosensitizer composition and light showed no ill effects of the treatment during the two days they lived before sacrifice. At necropsy, the atherosclerotic rabbit had no gross damage visible in the illuminated aortic section or surrounding tissue. By contrast, the normal rabbit had some minor damage visible in the para-aortic muscle, consisting of hemorrhage and purpura. Without being bound by theory, it is hypothesized that this damage was caused because the thickness of the normal artery was much less than the atherosclerotic aorta, and consequently, much of the light penetrated the artery and illuminated the surrounding tissue. The atherosclerotic rabbit that received light, but no conjugate was associated with any change to artery or surrounding tissue.

Histological examination of the arteries (Figure 15B. Top panel: histopathology of PDT treated atherosclerotic aorta; Bottom panel: histopathology of atherosclerotic aorta that received light but no conjugate) showed changes in the illuminated section of the atherosclerotic rabbit that received both conjugate and light, consistent with PDT effects in the targeted tissue. There was evidence of apoptosis (pyknotic nuclei) and an inflammatory infiltrate in the plaque (Figure 15B, left panel), together with some coagulative necrosis (Figure 15B, middle panel), and extravasated erythrocytes that may have come from the vasa vasorum and visible damage in the plaque (Figure 15B, right panel). Together, these histological data indicate that the treatment produced favorable modifications of plaque histology and reduced vulnerability. Histological changes were not observed in the normal rabbit that received photosensitizer composition and light, nor were any changes observed in the atherosclerotic rabbit that received light but no conjugate.

This technology satisfies the clear need for a new therapy that allows localized stabilization of vulnerable plaques in coronary arteries with the consequent reduced risk of rupture.

We claim

1. A method of stabilizing a vulnerable plaque in a subject comprising the steps of:
 - a) administering a therapeutically effective amount of at least one photosensitizer composition, wherein the photosensitizer composition is localized to the vulnerable plaque; and
 - b) light activating the photosensitizer composition to produce a phototoxic species; and
 - c) stabilizing the vulnerable plaque against rupture.
2. The method of claim 1, wherein the vulnerable plaque comprises inflammatory components, a large lipid pool, and a thin fibrous cap.
3. The method of claim 2, wherein the thin fibrous cap is less than about 150 microns thick.
4. The method of claim 2, wherein the thin fibrous cap is between less than about 100 microns thick.
5. The method of claim 2, wherein inflammatory components are selected from the group consisting of inflammatory cells, lipids, procoagulants and agents that promote inhibition of extracellular matrix production or degradation of extracellular matrix.
6. The method of claim 5, wherein the inflammatory cells are selected from the group consisting of smooth muscle cells, leukocytes, lymphocytes, monocytes, macrophages, foam cells, mast cells, endothelial cells, platelets, erythrocytes and polymorphonuclear cells.
7. The method of claim 6, wherein the lymphocytes comprise B-lymphocytes and T-lymphocytes.
8. The method of claim 6, wherein the polymorphonuclear cells comprise granulocytes and neutrophils.
9. The method of claim 6, wherein the inflammatory cells comprise greater than about 10% macrophages and/or monocytes.
10. The method of claim 6, wherein the inflammatory cells comprises greater than about 25% macrophages and/or monocytes.
11. The method of claim 2, wherein the lipid content is greater than about 10%.

12. The method of claim 2, wherein the lipid content is greater than about 25%.
13. The method of claim 1, wherein the photosensitizer composition comprises a photosensitizer coupled to a macromolecular carrier.
14. The method of claim 13, wherein the macromolecular carrier targets inflammatory components selected from the group consisting of inflammatory cells, lipids, procoagulants and agents that promote inhibition of extracellular matrix production or degradation of extracellular matrix.
15. The method of claim 14, wherein the inflammatory cells are selected from the group consisting of smooth muscle cells, leukocytes, lymphocytes, monocytes, macrophages, foam cells, mast cells, endothelial cells, platelets, erythrocytes and polymorphonuclear cells.
16. The method of claim 15, wherein the lymphocytes comprise B-lymphocytes and T-lymphocytes.
17. The method of claim 15, wherein the polymorphonuclear cells comprise granulocytes and neutrophils.
18. The method of claim 13, wherein the macromolecular carrier is selected from the group consisting of serum proteins, receptor ligands, microspheres, liposomes, antibodies, growth factors, peptides, hormones and lipoproteins.
19. The method of claim 13, wherein the macromolecular carrier binds to a scavenger receptor.
20. The method of claim 19, wherein the macromolecular carrier is selected from the group consisting of maleylated albumin, daunorubicin, doxorubicin, oxidized low density lipoprotein, acetylated low density lipoprotein, oxidized high density lipoprotein, malondialdehyde treated proteins, formaldehyde treated albumin, glycated albumin, polyinosinic acid, glycated lipoproteins, dextran sulfate, anionic phospholipids, fucoidin, carrageenan, polyvinyl sulfate and monoclonal antibodies that recognize CD11b, CD11c, CD13, CD14, CD16a, CD32 or CD68.
21. The method of claim 20, wherein the anionic phospholipid is phosphatidyl serine.

22. The method of claim 13, where in the macromolecular carrier targets the photosensitizer composition to a T cell.
23. The method of claim 22, where in the macromolecular carrier is selected from the group consisting of monoclonal antibodies that recognize CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD25, CD28, CD44 and CD71 and transferrin.
24. The method of claim 13, where in the macromolecular carrier targets the photosensitizer composition to the lipids comprising the lipid pool of the atheroma.
25. The method of claim 24, wherein the macromolecular carrier comprises a hydrophobic vehicles selected from the group consisting of liposomes, cremaphor EL, PEG/solvent mixtures, iodized castor oil, nanoparticles and micellar preparations.
26. The method of claim 25, wherein the liposome contains cholesterol.
27. The method of claim 25, wherein the liposome contains cardiolipin.
28. The method of claim 13, wherein the macromolecular carrier targets the photosensitizer composition to macrophages.
29. The method of claim 28, wherein the macromolecular carrier targets the photosensitizer composition to a macrophage biomolecule selected from the group consisting of tenascin C, tissue factor, tissue inhibitor of MMP 1, tissue inhibitor of MMP 2, oxidized LDL receptor, heme oxygenase-1, human cartilage gp-39, IL-6, IL-6 receptor, IL-10, IL-10 receptor, lectin-like oxidized LDL-receptor, monocyte inflammatory protein-1, monocyte inflammatory protein-1 receptor and macrophage chemoattractant protein-1 receptor.
30. The method of claim 22, wherein the macromolecular carrier targets the photosensitizer composition to a T cell biomolecule selected from the group consisting of IL-10, IL-10 receptor, monocyte inflammatory protein-1, monocyte inflammatory protein-1 receptor and transferrin.
31. The method of claim 13, wherein the macromolecular carrier targets the photosensitizer composition to foam cells.
32. The method of claim 13, wherein the macromolecular carrier that targets the photosensitizer composition a protease that degrades extracellular matrix.

33. The method of claim 32, wherein the protease is a metalloproteinase.
34. The method of claim 32, wherein the macromolecular carrier is a monoclonal antibody that binds to an epitope on a protease.
35. The method of claim 1, wherein the light activating the photosensitizer composition to produce a phototoxic species is administered in an amount sufficient to induce apoptosis and not necrosis of the cells comprising the vulnerable plaque.
36. A method of stabilizing a vulnerable plaque in a subject comprising the steps of:
 - a) administering a therapeutically effective amount of at least one photosensitizer composition comprising a photosensitizer coupled to a macromolecular carrier; and
 - b) localizing the photosensitizer composition to a vulnerable plaque consisting of inflammatory components, a large lipid pool, and a thin fibrous cap that is less than about 150 microns thick; and
 - b) light activating the photosensitizer composition to produce a phototoxic species; and
 - c) stabilizing the vulnerable plaque against rupture.
37. The method of claim 36, wherein the wherein the light activating the photosensitizer composition to produce a phototoxic species is administered in an amount sufficient to induce apoptosis and not necrosis of the cells comprising the vulnerable plaque.
38. A method of stabilizing a vulnerable plaque in a subject comprising the steps of:
 - a) administering a therapeutically effective amount of at least one photosensitizer composition comprising a photosensitizer coupled to a macromolecular carrier; and
 - b) localizing the photosensitizer composition to a vulnerable plaque consisting of inflammatory components, a large lipid pool, and a thin fibrous cap that is less than about 150 microns thick; and
 - c) light activating the photosensitizer composition to produce a phototoxic species, and wherein the light further produces cross links in the fibrous cap; and

- d) stabilizing the vulnerable plaque against rupture.
39. The method of claim 38, wherein the wherein the light activating the photosensitizer composition to produce a phototoxic species is administered in an amount sufficient to induce apoptosis and not necrosis of the cells comprising the vulnerable plaque.
40. A method of detecting a vulnerable plaque in a subject comprising the steps of:
- a) administering a fluorescent composition; and
 - b) localizing the composition to the vulnerable plaque; and
 - c) light activating the composition to illuminate the vulnerable plaque; and
 - d) identifying the vulnerable plaque.
41. The method of claim 40, wherein the fluorescent composition comprises a photosensitizer coupled to a coupled to a macromolecular carrier.
42. The method of claim 40, wherein the fluorescent composition comprises a fluorescent dye coupled to a macromolecular carrier.
43. The method of claim 40, wherein the fluorescent composition comprises a photoactive dye coupled to a macromolecular carrier.
44. A method of detecting a vulnerable plaque in a subject comprising the steps of:
- a) administering a detectable amount of at least one fluorescent composition, wherein the fluorescent composition is localized to a vulnerable plaque; and
 - b) light activating the vulnerable plaque to produce a fluorescent species; and
 - c) identifying the vulnerable plaque.
45. The method of claim 44, wherein the fluorescent composition comprises a photosensitizer coupled to a coupled to a macromolecular carrier.
46. The method of claim 44, wherein the fluorescent composition comprises a fluorescent dye coupled to a macromolecular carrier.
47. The method of claim 44, wherein the fluorescent composition comprises a photoactive dye coupled to a macromolecular carrier.

48. A method of detecting and treating a vulnerable plaque in a subject comprising the steps of:
- a) administering a detectable amount of at least one fluorescent composition, wherein the fluorescent composition is localized to a vulnerable plaque; and
 - b) administering a therapeutically effective amount of at least one photosensitizer composition, wherein the photosensitizer composition is localized to a vulnerable plaque; and
 - c) light activating the vulnerable plaque to produce a fluorescent species; and
 - d) identifying the vulnerable plaque; and
 - e) light activating the photosensitizer composition at the site of the vulnerable plaque to produce a phototoxic species; and
 - f) stabilizing the vulnerable plaque against rupture.
49. The method of claim 41, further comprising the steps of:
- d) light activating the photosensitizer at the site of the vulnerable plaque to produce a phototoxic species; and
 - e) stabilizing the vulnerable plaque against rupture.
50. The method of claim 43, further comprising the steps of:
- d) light activating the photoactive dye at the site of the vulnerable plaque to produce a phototoxic species; and
 - e) stabilizing the vulnerable plaque against rupture.
51. The method of claim 45, further comprising the steps of:
- d) light activating the photosensitizer at the site of the vulnerable plaque to produce a phototoxic species; and
 - e) stabilizing the vulnerable plaque against rupture.
52. The method of claim 47, further comprising the steps of:
- d) light activating the photoactive dye at the site of the vulnerable plaque to produce a phototoxic species; and
 - e) stabilizing the vulnerable plaque against rupture.
53. The method of claim 1, wherein the photosensitizer is chlorin_{e6}.

54. The method of claim 53, wherein the light is administered in a 20-500 J/cm dose.
55. The method of claim 53, wherein the light is administered in a 50-300 J/cm dose.
56. The method of claim 53, wherein the light is administered in a 100-200 J/cm dose.
57. The method of claim 36, wherein the photosensitizer is chlorin_{e6}.
58. The method of claim 57, wherein the light is administered in a 20-500 J/cm dose.
59. The method of claim 57, wherein the light is administered in a 50-300 J/cm dose.
60. The method of claim 57, wherein the light is administered in a 100-200 J/cm dose.
61. The method of claim 38, wherein the photosensitizer is chlorin_{e6}.
62. The method of claim 61, wherein the light is administered in a 20-500 J/cm dose.
63. The method of claim 61, wherein the light is administered in a 50-300 J/cm dose.
64. The method of claim 61, wherein the light is administered in a 100-200 J/cm dose.
65. The method of claim 48, wherein the photosensitizer composition comprises chlorin_{e6}.
66. The method of claim 65, wherein the light activating the photosensitizer composition is administered in a 20-500 J/cm dose.
67. The method of claim 65, wherein the light the activating the photosensitizer composition is administered in a 50-300 J/cm dose.
68. The method of claim 65, wherein the light activating the photosensitizer composition is administered in a 100-200 J/cm dose.
69. The method of claim 49, wherein the photosensitizer is chlorin_{e6}.
70. The method of claim 69, wherein the light activating the photosensitizer is administered in a 20-500 J/cm dose.

71. The method of claim 69, wherein the light activating the photosensitizer is administered in a 50-300 J/cm dose.
72. The method of claim 69, wherein the light activating the photosensitizer is administered in a 100-200 J/cm dose.
73. The method of claim 50, wherein the photoactive dye is chlorin_{e6}.
74. The method of claim 73, wherein the light activating the photoactive dye is administered in a 20-500 J/cm dose.
75. The method of claim 73, wherein the light activating the photoactive dye is administered in a 50-300 J/cm dose.
76. The method of claim 73, wherein the light activating the photoactive dye is administered in a 100-200 J/cm dose.
77. The method of claim 51, wherein the photosensitizer is chlorin_{e6}.
78. The method of claim 77, wherein the light activating the photosensitizer is administered in a 20-500 J/cm dose.
79. The method of claim 77, wherein the light activating the photosensitizer is administered in a 50-300 J/cm dose.
80. The method of claim 77, wherein the light activating the photosensitizer is administered in a 100-200 J/cm dose.
81. The method of claim 52, wherein the photoactive dye is chlorin_{e6}.
82. The method of claim 81, wherein the light activating the photoactive dye is administered in a 20-500 J/cm dose.
83. The method of claim 81, wherein the light activating the photoactive dye is administered in a 50-300 J/cm dose.
84. The method of claim 81, wherein the light activating the photosensitizer composition is administered in a 100-200 J/cm dose.
85. The method of claim 41, wherein the photosensitizer is chlorin_{e6}.
86. The method of claim 85, wherein the light is administered in a dose that is less than about 10 J/cm.
87. The method of claim 85, wherein the light is administered in a dose that is less than about 5 J/cm.

88. The method of claim 85, wherein the light is administered in a dose that is less than about 1 J/cm.
89. The method of claim 43, wherein the photoactive dye is chlorin_{e6}.
90. The method of claim 89, wherein the light is administered in a dose that is less than about 10 J/cm.
91. The method of claim 89, wherein the light is administered in a dose that is less than about 5 J/cm.
92. The method of claim 89, wherein the light is administered in a dose that is less than about 1 J/cm.
93. The method of claim 45, wherein the photosensitizer is chlorin_{e6}.
94. The method of claim 93, wherein the light is administered in a dose that is less than about 10 J/cm.
95. The method of claim 93, wherein the light is administered in a dose that is less than about 5 J/cm.
96. The method of claim 93, wherein the light is administered in a dose that is less than about 1 J/cm.
97. The method of claim 47, wherein the photoactive dye is chlorin_{e6}.
98. The method of claim 97, wherein the light is administered in a dose that is less than about 10 J/cm.
99. The method of claim 97, wherein the light is administered in a dose that is less than about 5 J/cm.
100. The method of claim 97, wherein the light is administered in a dose that is less than about 1 J/cm.
101. The method of claim 48, wherein the fluorescent composition comprises is chlorin_{e6}.
102. The method of claim 101, wherein the light is activating the fluorescent composition is administered in a dose that is less than about 10 J/cm.
103. The method of claim 101, wherein the light is activating the fluorescent composition administered in a dose that is less than about 5 J/cm.
104. The method of claim 101, wherein the light activating the fluorescent composition is administered in a dose that is less than about 1 J/cm.

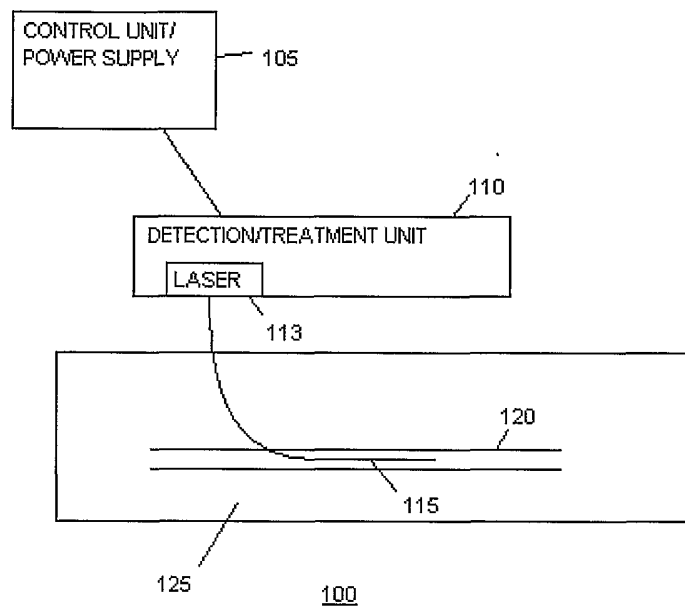
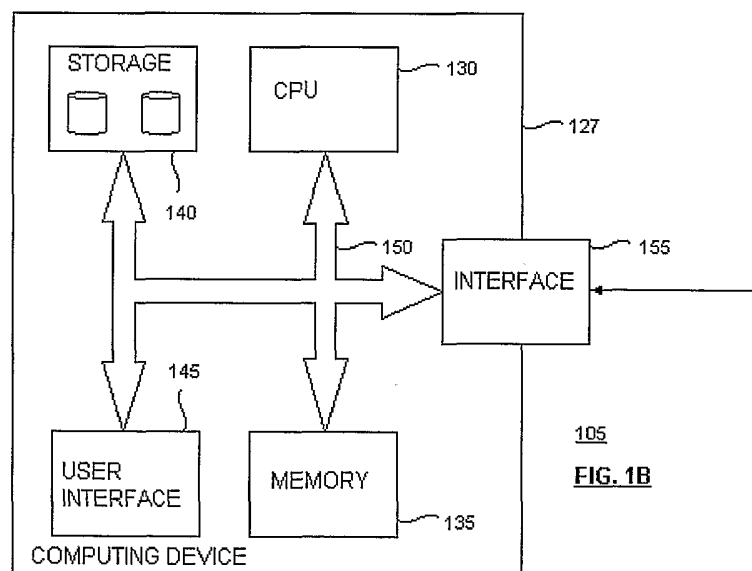
105. A method of detecting and treating a vulnerable plaque in a subject comprising the steps of:
- a) administering a detectable amount of at least one fluorescent composition, wherein the fluorescent composition is localized to a vulnerable plaque; and administering a therapeutically effective amount of at least one photosensitizer composition, wherein the photosensitizer composition is localized to a vulnerable plaque; and
 - b) light activating the vulnerable plaque to produce a fluorescent species; and
 - c) identifying the vulnerable plaque; and
 - d) light activating the photosensitizer composition at the site of the vulnerable plaque to produce a phototoxic species;
 - e) and stabilizing the vulnerable plaque against rupture.
106. A method of detecting and treating a vulnerable plaque in a subject comprising the steps of:
- a) administering a composition comprising a radiolabeled a macromolecular carrier; and
 - b) localizing the composition to the vulnerable plaque; and
 - c) measuring radioactive signal; and
 - d) identifying the vulnerable plaque; and
 - e) administering a therapeutically effective amount of at least one photosensitizer composition, wherein the photosensitizer composition is localized to the vulnerable plaque; and
 - f) light activating the photosensitizer composition at the site of the vulnerable plaque to produce a phototoxic species; and
 - g) stabilizing the vulnerable plaque against rupture.
107. An apparatus for detecting vulnerable plaque in a blood vessel, comprising: a light emitter operable to emit light having a first predetermined wavelength; and a light detector operable to detect light having a second predetermined wavelength, whereby a predetermined amount of at least one fluorescent

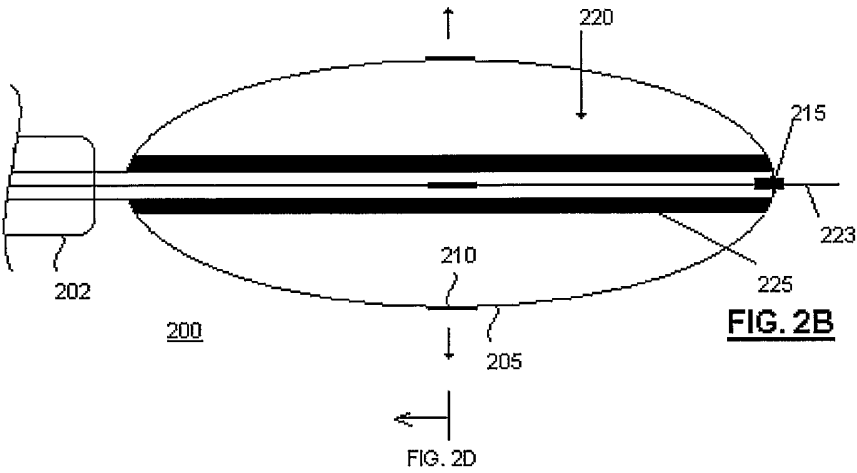
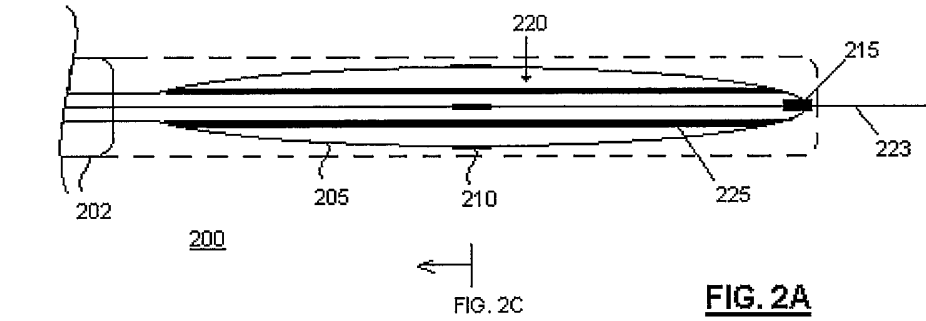
composition is administered to the blood vessel, the fluorescent composition localizes to the vulnerable plaque, and light having the first predetermined wavelength causes the fluorescent composition localized to the vulnerable plaque to emit light having the second predetermined wavelength.

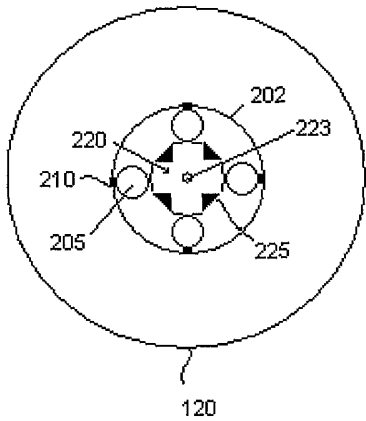
108. The apparatus of claim 107, wherein said light emitter and said light detector are included in a probe.
109. The apparatus of claim 108, wherein said probe is operable to be inserted into the blood vessel.
110. The apparatus of claim 107, further comprising a therapeutic light emitter operable to emit light having a third predetermined wavelength, whereby a therapeutically effective amount of at least one photosensitizer composition is administered to the blood vessel, the photosensitizer composition localizes to the vulnerable plaque, and light having the third predetermined wavelength causes the photosensitizer composition to produce a phototoxic species, which stabilizes the vulnerable plaque.
111. The apparatus of claim 110, wherein said therapeutic light emitter operable to emit light having the third predetermined wavelength at a predetermined power level, whereby light having the third predetermined wavelength at the predetermined power level causes the photosensitizer composition to produce the phototoxic species in an amount that does not induce necrosis of the cells comprising the vulnerable plaque.
112. The apparatus of claim 110, wherein the third predetermined wavelength is between 405 nm.
113. The apparatus of claim 107, wherein the first predetermined wavelength is 337 nm.
114. The apparatus of claim 107, wherein the second predetermined wavelength is between 666 nm and 668 nm.
115. The apparatus of claim 107, further comprising an inflatable vessel coupled to said light detector, whereby said light detector is moved towards a wall of the blood vessel by inflating said inflatable vessel.
116. The apparatus of claim 115, wherein said inflatable vessel contains saline.

117. The apparatus of claim 115, further comprising an external unit, whereby said inflatable vessel and said light detector are retractable into and extendible out from said external unit.
118. The apparatus of claim 107, further comprising said external unit.
119. The apparatus of claim 118, further comprising an elastic structure coupled to said light detector, said elastic structure and said light detector being retractable into and extendible out from said external unit, whereby said elastic structure moves said light detector towards a wall of the blood vessel when extended out from said external unit.
120. An apparatus for detecting and treating vulnerable plaque in a blood vessel, comprising:
a detector operable to detect an emission from the vulnerable plaque; and a light emitter operable to emit light having a predetermined wavelength, whereby light having the predetermined wavelength causes a photosensitizer composition administered and localized to the vulnerable plaque to produce a phototoxic species, stabilizing the vulnerable plaque.
121. The apparatus of claim 120, wherein the emission includes heat.
122. The apparatus of claim 120, wherein the emission includes radioactive signals from a composition comprising a radiolabeled macromolecular carrier administered and localized to the vulnerable plaque.

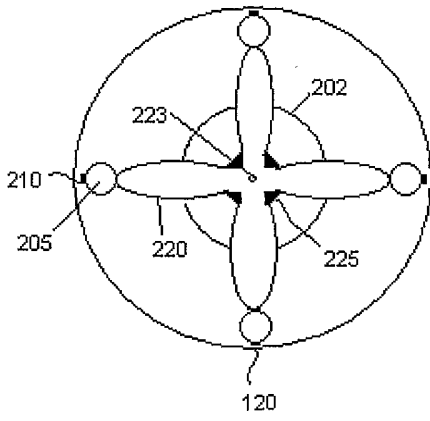
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**FIG. 1A****FIG. 1B**

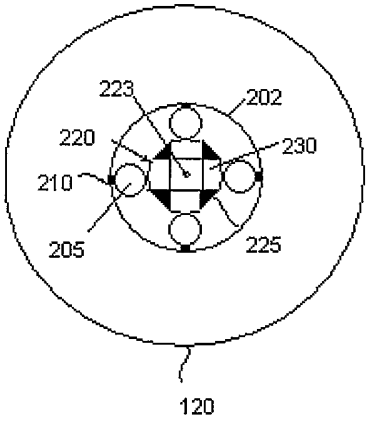




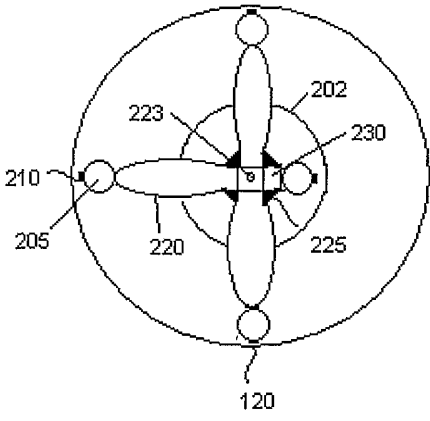
200
FIG. 2C



200
FIG. 2D



200
FIG. 2E



200
FIG. 2F

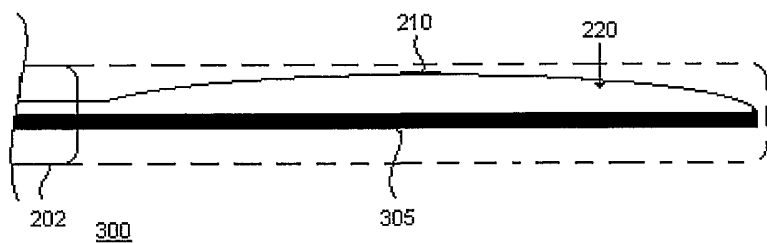


FIG. 3A

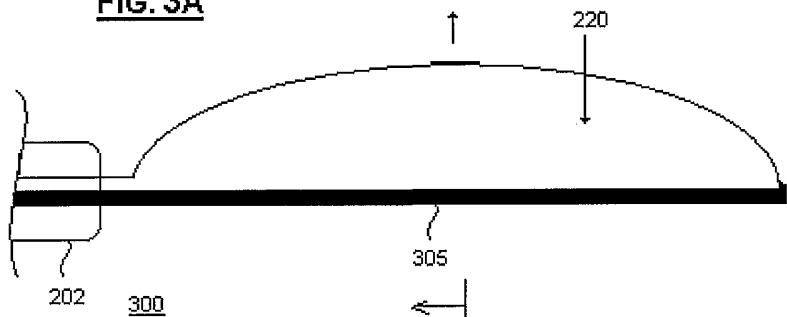


FIG. 3B

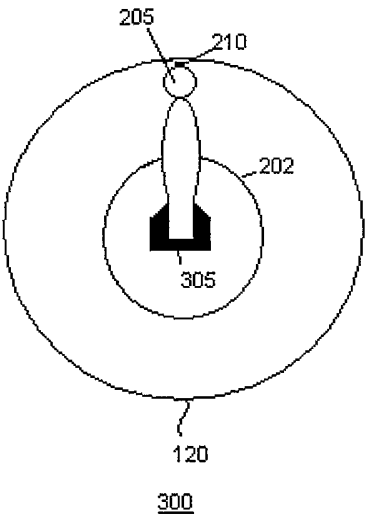
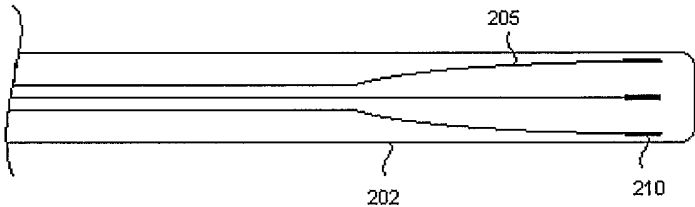
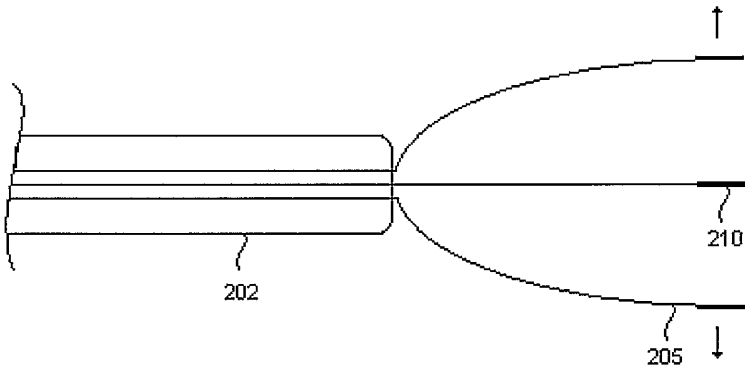


FIG. 3C



400
FIG. 4A



400
FIG. 4B

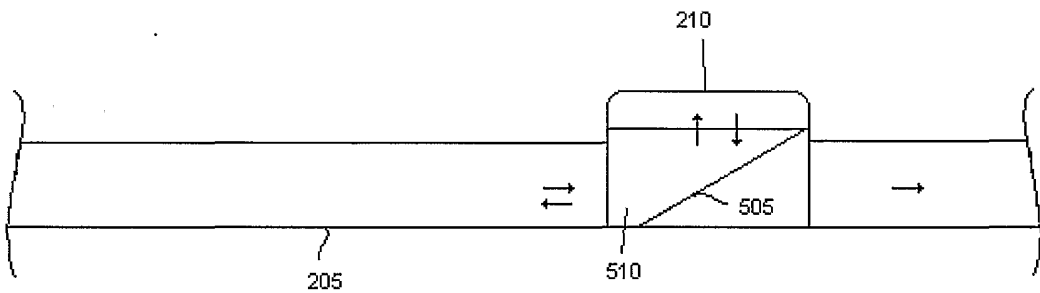


FIG. 5A

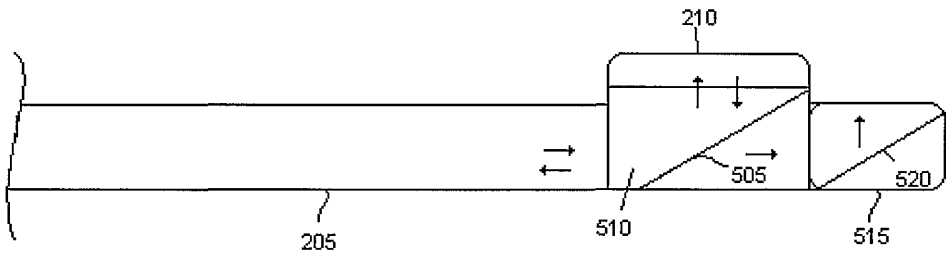


FIG. 5B

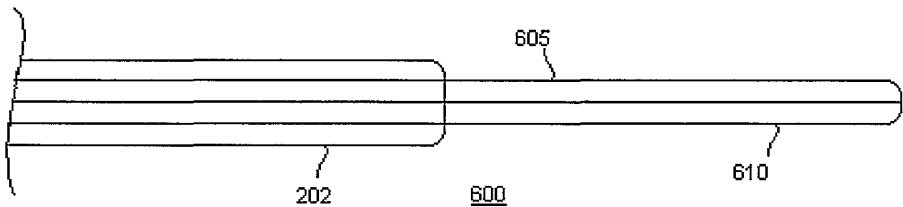


FIG. 6A

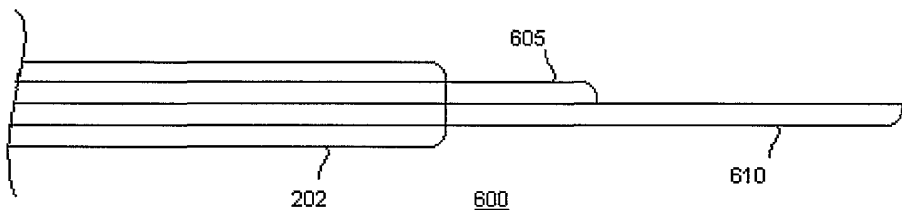


FIG. 6B

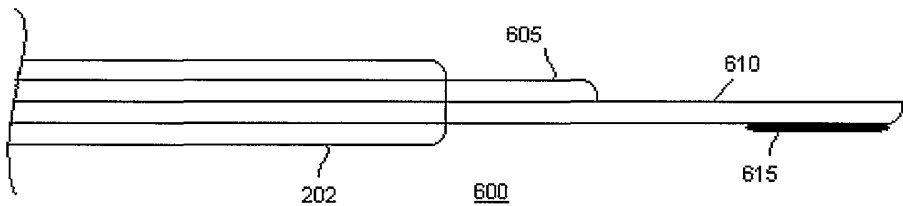
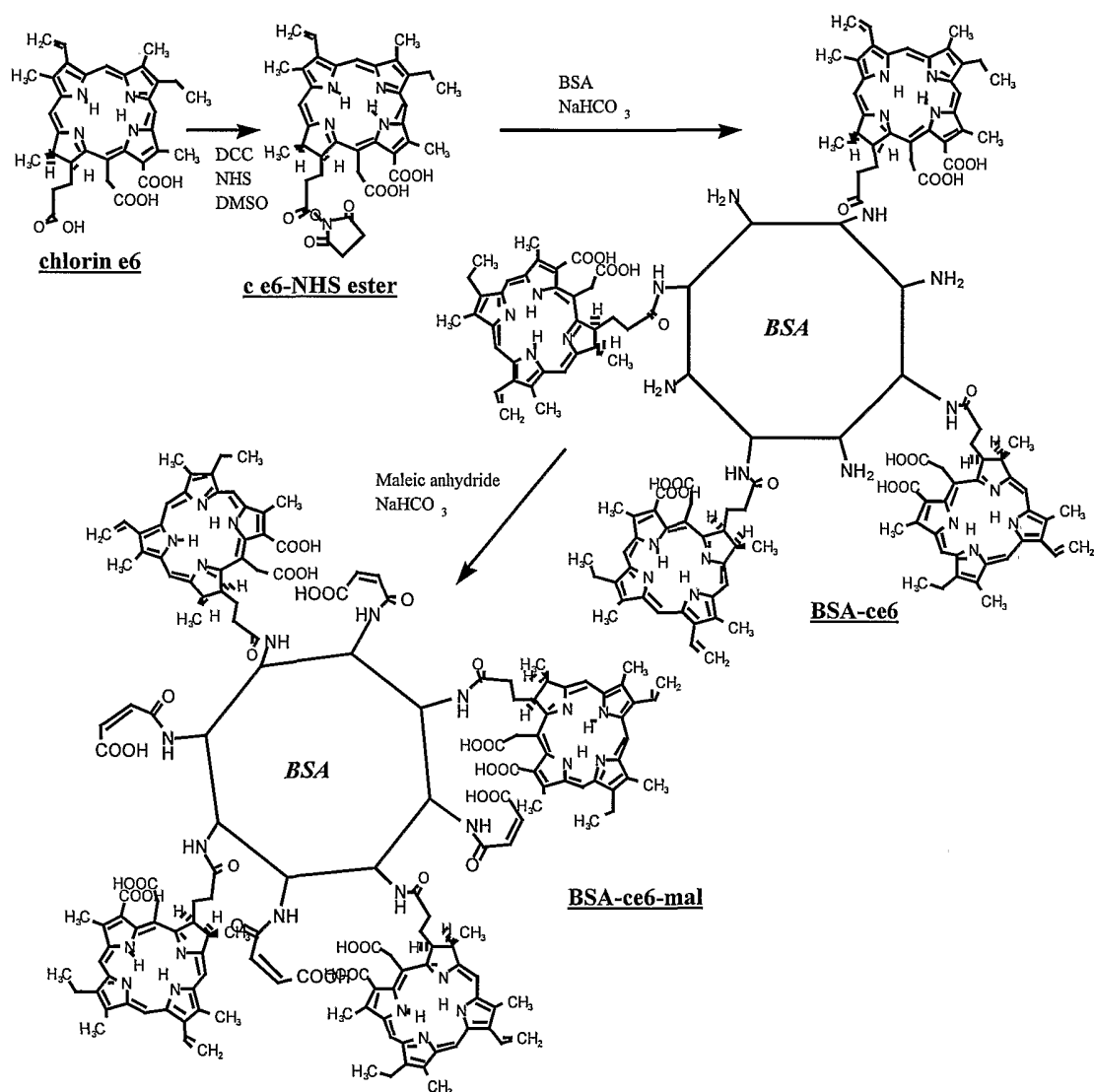


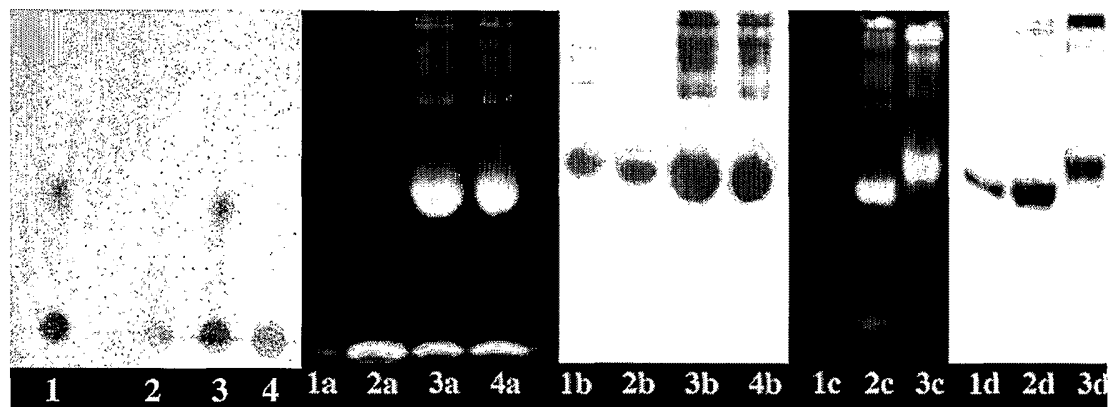
FIG. 6C

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Figure 7



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Figure 8

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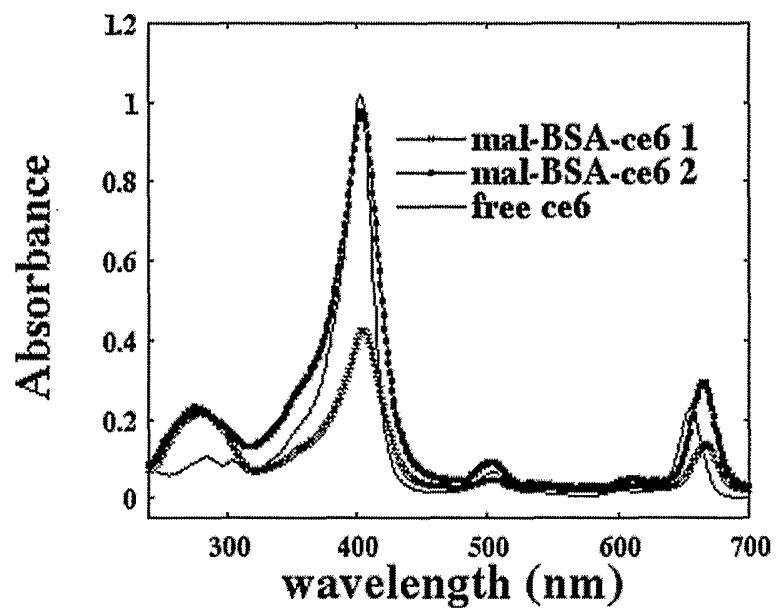
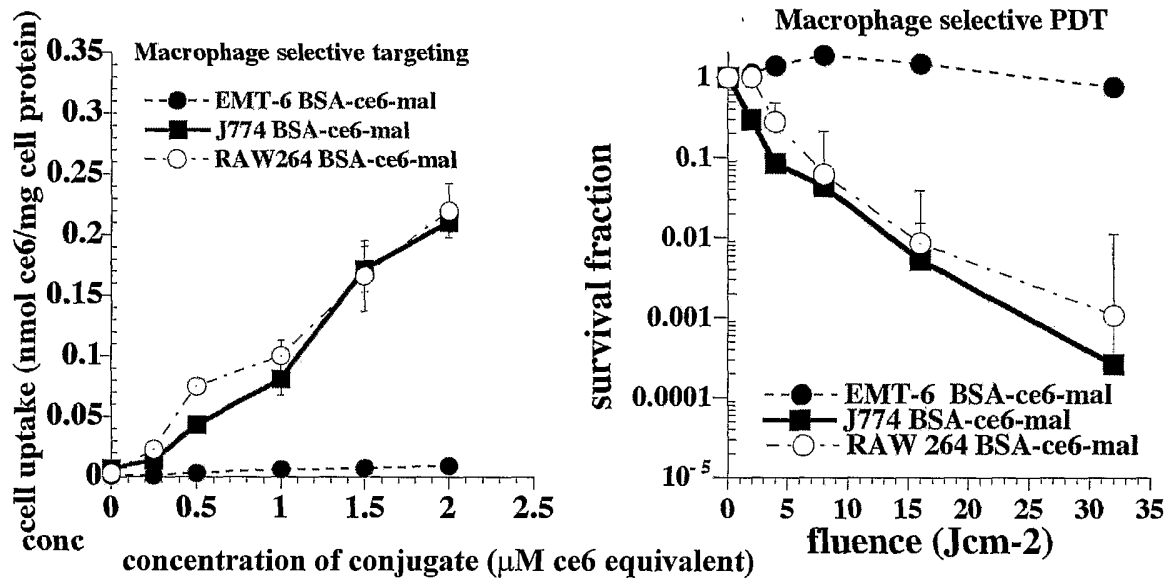


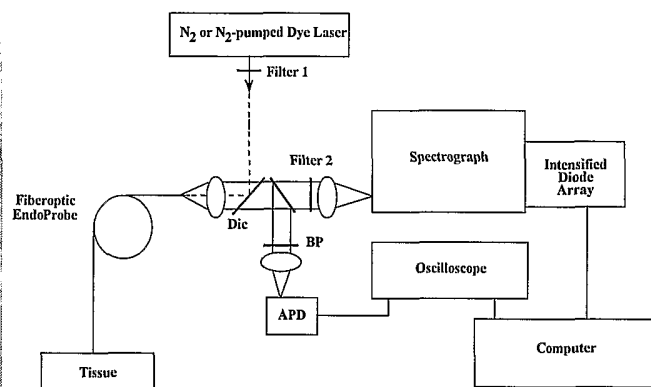
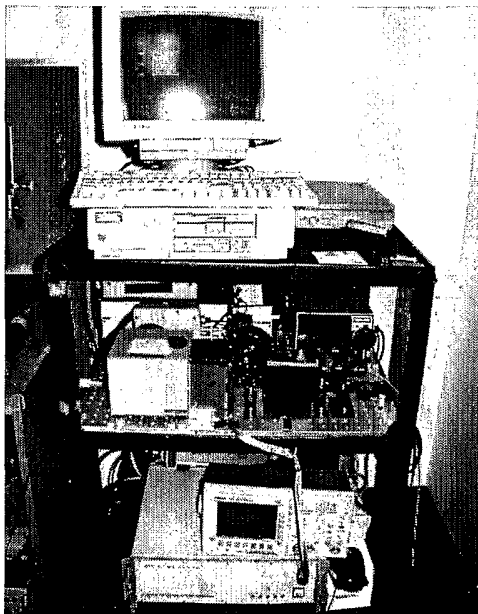
Figure 9

Figure 10



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Figure 11



Filter 1: UG-1 for 337 nm, none for Dye
Filter 2: O-51 for 337 nm, LL450 for Dye

Figure 12

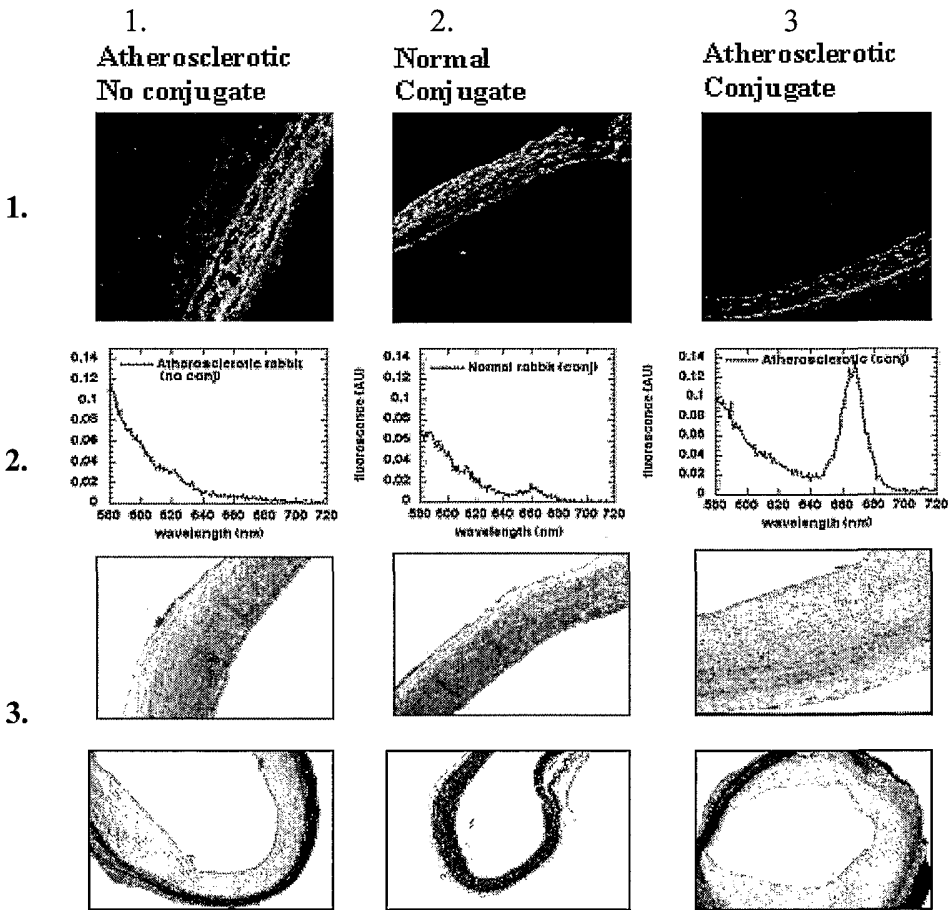


Figure 13

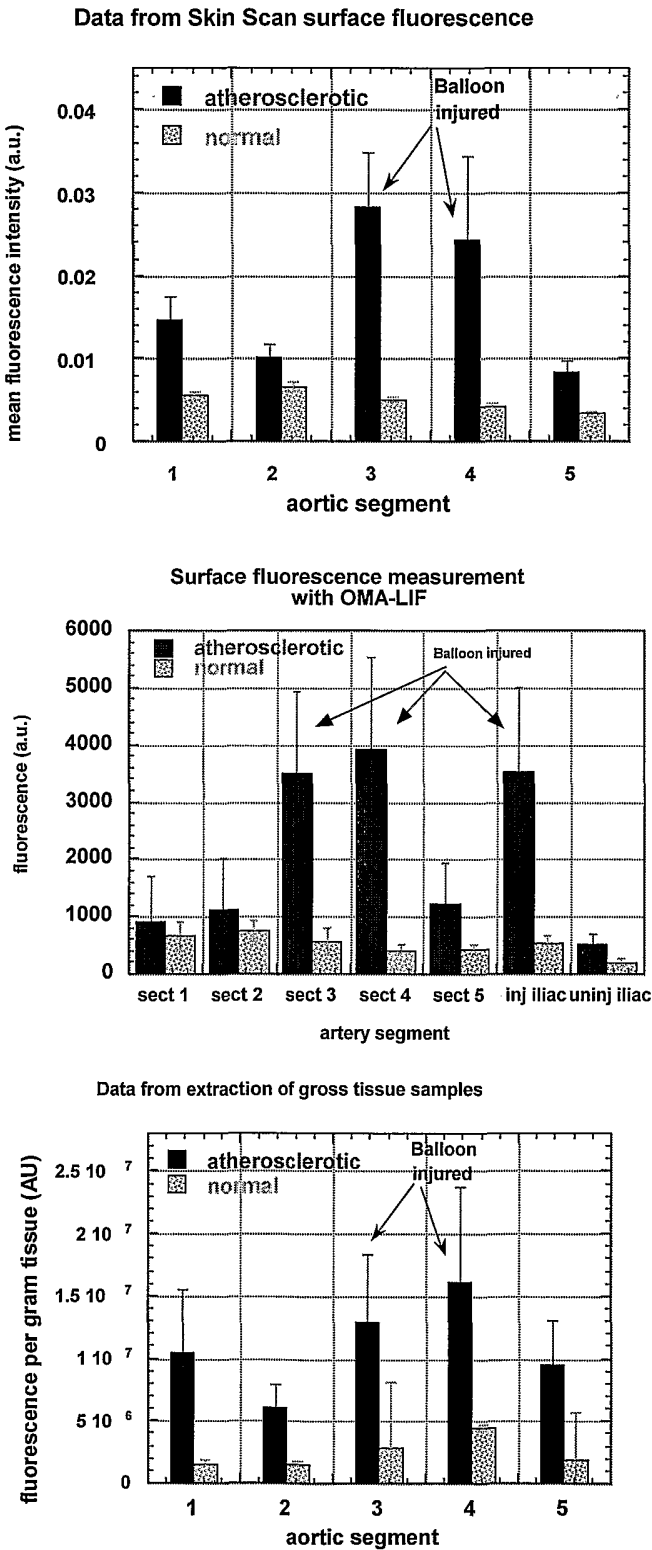
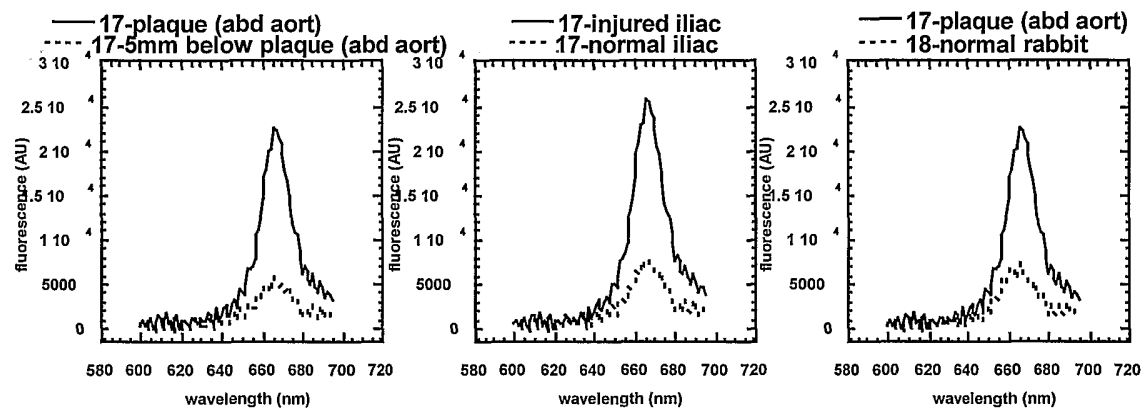
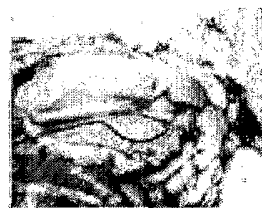


Figure 14



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Figure 15**A.****B.**

Pilot PDT experiment on 2 atherosclerotic animals (1 PDT treated, 1 that received light but no conjugate)

