Title: METHODS OF LOCALLY TREATING AND PREVENTING CARDIAC DISORDERS

Abstract: The present disclosure provides methods and compositions for treating coronary tissue damaged as a result of a cardiac disorder such as ischemia, acute myocardial infarction, vulnerable plaques, or reperfusion injury. Specifically, the cardiac disorder is treated using a locally-delivered therapeutic molecule.
METHODS OF LOCALLY TREATING AND PREVENTING CARDIAC DISORDERS

Related Application

The present application claims the benefit of U.S. Provisional Application No. 60/750,745 filed on December 15, 2005, which is incorporated herein by reference.

Background Of The Invention

One of the unmet clinical needs in interventional cardiology is amelioration or elimination of damage to heart tissue as a result of cardiac insult such as that caused by occluded coronary arteries – acute myocardial infarction (AMI). Not only does infarcted tissue decline due to lack of blood perfusion, but upon opening of the artery, the rapid reperfusion of the infarcted region also causes damage, inter alia cardiomyocyte apoptosis. In some instances, the long-term result of such damage is a permanent remodeling of ventricular tissue into a “scar” that is characterized by a thinning ventricular wall and expanded ventricular cavity. The scar region typically does not contain sufficient cardiomyocytes, vascular cells, fibroblasts, and/or nerve cells to sustain the normal pattern of depolarization propagation and/or contraction for efficiently pumping of blood. Reduced blood flow, associated with clinically measurable indications such as decreased contraction force, decreased ejection fraction, increased left ventricular (LV) volume, and increased LV wall stress, are the frequent sequela of AMI.

There are several clinical approaches to preventing AMI or reducing the damage caused by AMI, falling into three main categories: cell therapy; gene therapy; and drug treatment. Other approaches include, for example, the enhancement of the healing properties of blood through super-oxygenation or other ex-vivo blood treatment. Cell therapy includes surgically introducing cells directly into the heart muscle; injecting cells from inside the heart via catheter-mounted syringes using standard interventional techniques; introducing cells into a coronary artery, whence they would be carried by the blood downstream into the injured zone; or introducing cells intravenously. Gene therapy includes the local or systemic delivery of DNA, vectors, or viruses encoding genes intended to induce cardiac angiogenesis or myogenesis. Cell therapy approaches to AMI present many challenges including, for
example, the inefficient injection of cells whereby only a few of the injected cells remain at the site of injection; the poor survival of transplanted cells; the choice or selection of cell type that is the most beneficial; the expensive and time consuming processes for harvesting and \textit{ex vivo} expansion of autologous cells; and the complex clinical procedures required to introduce these cells into the heart of a patient. Likewise, gene therapy is an impractical approach due to low efficiency of delivery, the inability to precisely control dose, potential toxicity in non-target tissues, and difficulties in choosing appropriate gene constructs.

Two major categories of drug treatment for AMI may be defined. The first, anti-thrombotic, or thrombolytic, therapy reduces or eliminates thrombi that occlude the coronary arteries and cause hypoxia. Examples include: fibrinolytic agents such as intravenous streptokinase or tissue plasminogen activator; anti-coagulant agents such as aspirin, heparin, and factor Xa inhibitors; platelet inhibitors (GP IIb/IIIa receptor inhibitors) such as abciximab and tirofiban; and inhibitors of adenosine diphosphate stimulated platelet aggregation such as clopidogrel (Antman and Van de Werf, \textit{Circulation}, 109:2480-2486, 2004). In recent years treatment regimens have been developed and widely used, typically consisting of combinations of the above therapeutic agents, both to dissolve occluding thrombi which have formed in coronary arteries (often being the root cause of myocardial infarctions) and for the prevention of subsequent thrombi during and after percutaneous transluminal coronary angioplasty (PTCA) procedures and stent implantation.

The second category of drug treatments for AMI includes pharmacological modulators of AMI (PMAMI). This category consists of therapies intended to accomplish one or more of the following: reduce the size and extent of myocardial damage due to coronary artery occlusion; prevent or ameliorate injury due to reperfusion of the infarcted or ischemic zone; reduce post-reperfusion ventricular remodeling; enhance regeneration of damaged tissue; promote endothelialization; and reverse or eliminate secondary diseases resulting from AMI such as cardiac fibrosis. Such therapies may target specific modes of action such as anti-apoptosis, modulation of differentiation, cardio-protection, angiogenesis, or immunomodulation. Drug treatments from this category, that are intended to ameliorate or prevent the effects of AMI, are usually administered in conjunction with an anti-thrombosis drug regimen.
Ischemically damaged blood vessels are prone to endothelial damage, including activation of the complement system and deposition of complement factors such as C1q and the terminal complement complex (TCC). This is particularly apparent upon post-ischemic reperfusion, and is manifested by shedding of the anti-inflammatory and anti-coagulant heparin sulfate proteoglycan (HSPG) layer, a component of the glycocalyx, which includes glycoproteins, proteoglycans, and associated glycosaminoglycans. This process of shedding all or part of the HSPG layer typically results in increased inflammation, coagulation followed by thrombosis, and eventual myocardial damage leading to infarction.

In addition, it has been reported that, local (intravascular) administration of the glycosaminoglycan analog dextran sulfate reduces the ischemic damage caused by coronary artery occlusion (Banz et al, Eur Heart J, 0:4211, 2005).

Summary Of The Invention

The present invention offers an improvement in pharmacological modulators or PMAMI. In a specific embodiment the improvement offers locations and methods for the local or regional delivery of PMAMI. The target regions consist of particular areas of the heart that have undergone ischemic damage, inflammation, necrosis, and/or infarction, and the coronary arteries. In particular, an artery that has undergone occlusion leading to ischemia is a prime local or regional delivery route because that same artery will deliver PMAMI directly to the damaged coronary region served by that artery. Alternatively, PMAMI will deliver to upstream regions of the coronary arteries, near their junction with the aorta, from whence all of the coronary arteries and the served coronary tissue downstream receives the delivered therapeutic pharmacological modulators.

Note is made of amelioration of damage to the HSPG layer by administering appropriate components of the glycocalyx, such as glycoproteins, proteoglycans, glycosaminoglycans, or analogs thereof. Such compounds are suitable to formulation into microparticles, using known methods such as the stable polymer aqueous/aqueous emulsion system described in U.S. Patent 6,805,879. In turn, such microparticles could contain or encapsulate PMAMI. Thus, in addition to providing a beneficial effect on the damaged HSPG layer upon dissolution, microparticles made from glycosaminoglycan analogs would also
serve the additional function of delivering therapeutic molecules to the site of coronary ischemic damage. It has been reported that such glycosaminoglycan analogs particularly bind to regions or sites in which the glycocalyx has been damaged. This characteristic would further target microparticles containing PMAMI to damaged regions. In an embodiment of the present invention microparticles made of or coated with glycosaminoglycans, carrying PMAMI, will bind to and accumulate at damaged areas, thus delivering PMAMI preferentially to those damaged areas.

Atherosclerosis often manifests in lipid-containing plaques in the coronary arteries that are non-occlusive (and thus difficult to detect) and thin-capped (and thus prone to rupture). Such plaques are referred to as "vulnerable plaques" and are considered to be particularly dangerous because rupture usually leads to thrombosis which in turn often leads to myocardial infarction or death. Such regions of vulnerable plaque (VP) also display damaged or absent glycocalyx components, and are thus subject to amelioration by treatment with microparticles made of glycosaminoglycan polymers containing therapeutic compounds.

In this case, pharmaceutical modulators of vulnerable plaque (PMVP) would be intended to promote healing and/or prevent rupture of the plaque. Potential modes by which PMVP could accomplish this are, e.g., reducing inflammation, inhibiting the breakdown of collagen in the fibrous cap, and/or enhancing endothelialization of the plaque region. In this regard, certain PMAMI, such as enhancers of endothelialization and inhibitors of inflammation, would also serve as PMVP. VP is difficult to detect and expensive to treat, largely because both detection and treatment will most likely require an interventional procedure, which in itself is expensive and not without risk. Thus, treatment of VP with microparticles that are locally or regionally delivered into the arterial lumen upstream of the suspected VP location would serve as a practical, economical, and efficacious treatment for VP. Such treatment would likely be given during the course of an existing interventional procedure, thus obviating the increased risk and expense of additional interventions for VP treatment.

This invention comprises the use of therapeutic microparticles produced from glycosaminoglycan (GAG) polymers that are locally delivered serves the multiple purposes of: delivering PMAMI and/or PMVP to the region of damage or disease; delivering the glycocalyx-healing GAG to the region of damage; and enabling efficient and precise local or
regional delivery by, e.g., intra-arterial injection or stent-based delivery during the course of PTCA. An additional level of targeting these microparticles to the site of damage/disease may also be achieved by the inclusion of antibodies or antibody fragments in the microparticle or its coating. Such antibodies would bind to molecular markers that are characteristic of and specific to regions of AMI and/or VP.

Accordingly, it is an object of this invention to provide improved methods and compositions for the treatment of cardiac disorders, including acute myocardial infarction and vulnerable plaque.

The present in disclosure provides methods and compositions for treating cardiac disorders. In one aspect, the invention provides a method for treating a cardiac disorder in a patient by locally administering to the patient a therapeutic molecule (e.g., a PMAMI). In a related aspect, the invention provides an implantable device comprising a therapeutic molecule capable of treating a cardiac disorder. In either of the foregoing aspects, the therapeutic molecule is, in particular embodiments and without limitation, an estrogen (e.g., 17β-estradiol), a prostaglandin EP₃ receptor agonist, a caspase inhibitor, a potassium channel opener, a nitric oxide donor (e.g., nicorandil), an aldosterone receptor antagonist (e.g., spironolactone and eplerenone), a compound that blocks platelet-endothelial cell adhesion molecules (e.g., PECAM-1), IL-6/sIL-6R or IL-6, a GP130 agonist, an IL-18 antagonist, a glycosaminoglycan analog (e.g., dextran sulfate), a plasminogen activator inhibitor-1 antagonist, relaxin, clusterin, adiponectin, a p38 MAP kinase inhibitor (e.g., SB203580), a cardiac regeneration factor (e.g. CRF-1 and CRF-2), insulin-like growth factor 1 (IGF-1), hepatocyte growth factor (HGF), erythropoietin (EPO), carbamylated EPO (CEPO), or tissue-protective cytokines (TCPs).

Cardiac disorders amenable to treatment by this method include, for example, acute myocardial infarction, a chronic ischemic condition, reperfusion injury, chronic heart disease, vulnerable plaques, and cardiac fibrosis. Suitable molecules for use in this method include, for example, 17β-estradiol, and estrogen receptor agonists.

In some embodiments, a therapeutic molecule is locally administered by any appropriate route or using any appropriate technique including, for example, intravenous or
intra-arterial injection, during percutaneous transluminal coronary angioplasty (including delivery using a PCTA balloon), and via an implantable device (e.g., a stent). A therapeutic molecule, in particular instances, is present as a free base, salt, bound as a conjugate to another molecule, or encapsulated (e.g., within biodegradable microparticles). Particular note is made of the method wherein microparticles display on their surface antibodies, antibody fragments or peptides that recognize molecular markers selected from the group consisting of MMP-1, MMP-8, MMP-13, fibronectin, osteopontin, TLR4, and IL1RL1-b.

The invention further comprises an implantable device comprising a cardiotherapeutic microparticle consisting of a glycosaminoglycan matrix containing a therapeutic molecule selected from the group consisting of a prostaglandin EP3 receptor agonist, a caspase inhibitor, a potassium channel opener, a nitric oxide donor, an aldosterone receptor antagonist, an inhibitor of platelet-endothelial cell adhesion molecules, IL-6/sIL-6R, IL-6, a GP130 agonist, an IL-18 antagonist, a glycosaminoglycan analog, a plasminogen activator inhibitor-1 antagonist, relaxin, clusterin, adiponectin, p38 MAP kinase inhibitor, a cardiac regeneration factor, insulin-like growth factor 1 (IGF-1), hepatocyte growth factor (HGF), erythropoietin (EPO), carbamylated EPO (CEPO), and tissue-protective cytokines (TCPs). In some embodiments the microparticle is coated on the device, and further wherein the microparticle is contained within a polymer coating on the device such as a stent.

This invention further comprises a method for treating a cardiac disorder in a patient, said method comprising locally administering a cardiotherapeutic microparticle consisting of a glycosaminoglycan matrix containing a therapeutic molecule selected from the group consisting of an estradiol, an inhibitor of MMP-1/collagenase-1, MMP-8/collagenase-2 or MMP-13/collagenase-3, dexamethasone, FK506, pimecrolimus, sirolimus, everolimus, biolimus A9, ABT578, AP23573, nicorandil, an IL-18 antagonist, a VEGF inhibitor, a legumain inhibitor, a Lp-PLA2 inhibitor, a cathepsin S inhibitor, ghrelin, angiotatin, endostatin, SU5416, ZD6474, and 2-methoxyestradiol. Particular note is made of the specific cardiac disorder of vulnerable plaque. In some applications of the method microparticles display on their surface antibodies, antibody fragments, or peptides that recognize molecular markers selected from the group consisting of CD40 ligand, lectinlike oxidized low-density lipoprotein receptor-1 (LOX-1), pregnancy-associated plasma protein A (PAPP-A),
monomeric (or modified) C-reactive protein (mCRP), fibrillar collagen types I and III, specifically cleaved interstitial collagen, MMP-1, MMP-8, MMP-13, \( \alpha_\beta_3 \) integrin, ADAMDEC1, JAM-A, RAGE, and vascular cell adhesion molecule-1 (VCAM-1).

In yet another embodiment the invention includes an implantable device comprising a cardiotherapeutic microparticle consisting of a glycosaminoglycan matrix containing a therapeutic molecule selected from the group consisting of an estradiol, an inhibitor of MMP-1/collagenase-1, MMP-8/collagenase-2 or MMP-13/collagenase-3, dexamethasone, FK506, pimecrolimus, sirolimus, everolimus, biolimus A9, ABT578, AP23573, nicorandil, an IL-18 antagonist, a VEGF inhibitor, a legumain inhibitor, a Lp-PLA\(_2\) inhibitor, a cathepsin S inhibitor, ghrelin, angiotatin, endostatin, SU5416, ZD6474, and 2-methoxyestradiol. Optionally in the practice of this method the microparticle is coated on the device, and further optionally wherein the microparticle is contained within a polymer coating. Particular note is made of the device being a stent.

By "cardiac disorders" is meant any disease or disorder of the cardiac tissue, particularly the cardiac muscle, associated with or caused by an ischemic condition, reduction in blood flow, physical trauma (e.g., associated with injury or a surgical procedure). Cardiac disorders include, but are not limited to, AMI, acute or chronic ischemic conditions, reperfusion injury, chronic heart disease (CHD), VP, and cardiac fibrosis.

By "coronary tissue" is meant the cardiac muscle, consisting of fused cardiomyocytes, and ancillary cell types normally present but found in much smaller numbers. Examples of such cell types are vascular cells (including endothelial and smooth muscle cells), fibroblasts and other connective tissue cells, neurons and other types of nerve cells, and progenitor cells such as cardiac stem cells (Beltrami et al., *Cell*, 114:763-776, 2003). Coronary tissue also includes the extracellular matrix and associated molecules surrounding the foregoing cellular components.

By "percutaneous transluminal coronary angioplasty (PTCA)" is meant a procedure for treating heart disease in which a catheter assembly having a balloon portion is introduced percutaneously into the cardiovascular system of a patient via the brachial or femoral artery. The catheter assembly is advanced through the coronary vasculature until the balloon portion
is positioned across the occlusive lesion. Once in position across the lesion, the balloon is inflated to a predetermined size to radially compress against the atherosclerotic plaque of the lesion to remodel the vessel. The balloon is then deflated to a smaller profile to allow the catheter to be withdrawn from the patient’s vasculature.

By an “effective amount,” in reference to a therapeutic compound or composition, is meant an amount of a compound or composition, alone or in a combination according to the invention, required to affect a therapeutic response (i.e., treatment of cardiac disorders). The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of cardiac disorders (e.g., AMI) varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending medical professional will decide the appropriate amount and dosage regimen. Such amount is referred to as an “effective” amount.

By “treating” is meant administering a pharmaceutical composition for the purpose of improving the condition of a patient by reducing, alleviating, or reversing at least one adverse effect or symptom.

“Locally” as to treatment shall mean injection via intra-arterial catheter into the lumen or wall of one or more coronary arteries and/or elution from a stent or other device that has been implanted in a coronary artery, such that therapeutic microparticles will be delivered to diseased regions of coronary arteries and/or cardiac tissue.

“Cardiotherapeutic microparticles” are a matrix including a combination of a PMAMI and/or a PMVP which is formulated with a GAG.

For convenience, a glossary of acronyms are presented below:

ACS acute coronary syndrome
AMI acute myocardial infarction
CHD chronic heart disease
GAG glycosaminoglycan
HSPG heparin sulfate proteoglycan
LV left ventricular
PTCA percutaneous transluminal coronary angioplasty
PMAMI pharmacological modulators of AMI
TCC terminal complement complex
VP vulnerable plaque.

Brief Description Of The Drawings

FIGURE 1 is a schematic diagram showing components of a targeted cardiotherapeutic microparticle system for the treatment of myocardial infarction and its effects. The formulated treatment is introduced by any convenient route (e.g., injection through a catheter into the artery feeding the infarcted zone and/or eluted from a stent placed in the artery feeding the infarcted zone and/or into the infarcted region of the cardiac wall from within the ventricle). Targets sites for PMAMI therapy include the infarcted zone and surrounding border regions that are served by the coronary artery(ies) that had previously been occluded.

FIGURE 2 is a schematic diagram showing the general structure of a specific delivery system for PMAMI and/or PMVP, i.e., cardiotherapeutic microparticles or nanoparticles in the size range of 20 – 2000 nm in diameter. Such particles consist of polymeric glycosaminoglycan analogs that serve as a matrix for PMAMI or PMVP (FIG 2a). Layers within the microparticles can be used to enhance the kinetics or utility of the system, such as a degradable polymer coating containing PMAMI / PMVP (FIG 2b). Additional disease-specific targeting can be achieved through the use of specific antibody coatings and/or GlcNAc conjugates which may additionally contain PMAMI / PMVP, shown as a degradable polymer coating containing PMAMI / PMVP and/or targeting antibodies and/or GlcNAc conjugates (FIG 2c). Targeting molecules such as antibodies or GlcNAc conjugates may also be entrained in the cardiotherapeutic microparticle itself, without an outer layer (FIG 2d).
Detailed Description Of The Invention

The present invention provides methods and compositions for treating coronary tissue damaged as a result of a cardiac disorder such as ischemia, AMI, reperfusion injury, and atherosclerosis. Specifically, the cardiac disorder is treated locally using pharmacological modulators of AMI (PMAMI) and pharmacological modulators of vulnerable plaque (PMVP) that have been formulated as microparticles, in which the microparticle matrix consists of GAGs or other materials that, in addition to providing a biodegradable matrix in which to deliver PMAMI, could serve to functionally restore the HSPG layer in damaged endothelium. Also disclosed are methods and compositions for targeting such microparticles to the sites of disease or damage, by virtue of (i) enhanced binding of GAGs to areas of missing or damaged HSPG layer and (ii) coating the microparticles with antibodies specific to the damaged or diseased locations. The treatment may be administered by any appropriate method including, for example, via an implantable stent, via a drug-delivery PTCA balloon, as an intra-arterially injected bolus during PTCA, or via intracoronary injection.

Pharmacological Modulators of Acute Myocardial Infarction

There are several specific target regions within the infarcted or diseased zone of the heart that are targets for PMAMI therapies. Without being bound by any particular theory it is believed that, for an established myocardial infarction, the scar resulting from ischemic cell death and healing is the least responsive to therapy because the endogenous cardiac tissue capable of functioning in coordination with the rest of the heart has been replaced by non-functional scar tissue cells and extracellular matrix. Tissue in areas surrounding the scar border regions is reported to be progressively more functional and thus more capable of generating, supporting, and incorporating new cells that form or support functional tissue. Tissue that has undergone ischemia, but has not yet undergone apoptosis, necrosis, or scarring, is another target for PMAMI therapy insofar as timely treatment (e.g. during a PTCA procedure taking place as soon as possible after the onset of symptoms), slows or reverses the decline of this tissue via ischemic or reperfusion injury.
There are a variety of classes of molecules (PMAMI) that may be used to reduce and/or reverse the damage caused by AMI or another cardiac disorder. Any of these PMAMI may be used in accordance with the principles of this disclosure. Examples of useful PMAMI include, for example: estrogen (e.g., 17β-estradiol), prostaglandin EP₃ receptor agonists, caspase inhibitors, potassium channel openers, nitric oxide donors (e.g., nicorandil), aldosterone receptor antagonists (e.g., spironolactone and eplerenone), compounds that block platelet-endothelial cell adhesion molecules (e.g., PECAM-1), IL-6/sIL-6R or IL-6, GP130 agonists, IL-18 antagonists, glycosaminoglycan analogs (e.g., dextran sulfate), plasminogen activator inhibitor-1 antagonists, relaxin, clusterin, adiponectin, inhibitors of p38 MAP kinase (e.g., SB203580), cardiac regeneration factors (e.g. CRF-1 and CRF-2), insulin-like growth factor 1 (IGF-1), hepatocyte growth factor (HGF), erythropoietin (EPO), carbamylated EPO (CEPO), and/or tissue-protective cytokines (TCPs). For the purpose of this disclosure, any of the above examples of PMAMI, as well as other PMAMIs, are candidates for formulation as PMAMI/GAG microparticles, or cardiotherapeutic microparticles.

Vascular Protective Effects of Estrogen

17β-estradiol (estrogen, E2) is an endogenous steroid hormone found in men and women, and is the predominant female sex hormone. Anti-atherogenic properties of estrogen have been reported. Numerous clinical and experimental studies have demonstrated that estrogen improves the lipid profile and has direct protective effects on the vasculature (Barrett-Connor, Circulation, 95:252-264, 1997; Stampfer et al, N Engl J Med, 325:756-762, 1991; Mendelsohn and Karas, N Engl J Med, 340:1801-1811, 1999). Gender differences in cardiovascular disease are well recognized (Farhat et al, FASEB J, 10:615-624, 1996). Cardiovascular disease is rare in premenopausal women and the Framingham Study suggested that prior to menopause the incidence of ischemic heart disease in women is considerably less than that of males (Lerner and Kannel, American Heart Journal, 111:383-390, 1986). The lower mortality from ischemic heart disease among premenopausal women is reported to be largely due to endogenous circulating estrogens and, when estrogen production subsides following the menopause, there is a sharp increase in mortality (Colditz et al, N Engl J Med, 316:1105-1110, 1987). Premature menopause from bilateral oophorectomy is reported as
associated with an increase in coronary artery disease (Barrett-Connor and Bush, *JAMA*, 265:1861-1867).

Local delivery of estradiol directly to the site of action for vascular and coronary disease, in accordance with the principles of this disclosure, will improve therapeutic outcome compared to systemic delivery. Reported advantages of local estradiol delivery include anti-atherogenic properties, inhibition of neointimal proliferation whilst enhancing endothelial repair, preservation of plaque structure, and direct myocardial effects.

Estrogen, and more particularly 17β-estradiol, is useful in the treatment of vascular disorders. Estradiol has been shown to inhibit SMC proliferation and migration. By contrast, estradiol enhances endothelial cell proliferation and migration which promotes endothelialization. New et al, *Catheterization and Cardiovascular Interventions*, 57: 266-271, 2002; Chandrasekar, *J. Invasive Cardiology*, 16: 719-722, 2004. Estradiol is also reported to have local effects on the vasculature including enhanced vascular dilation, increased NO production, and reduced platelet activity. Finally, estradiol is reported to produce anti-inflammatory effects by blocking the NFκB pathway (Galea et al, *Mol Neurosci*, 13:1469-1472, 2002) and protecting cells from proinflammatory cytokine effects (Contreras et al, *Transplantation*, 74:1252-1259, 2002). These effects are observed for 17β-estradiol in the sub-to-low nanomolar range.

The mechanisms for these physiological effects are multifactorial. Estrogen is believed to alter the atherogenic lipid profile and also have a direct action on blood vessel walls. Estrogen is reported to have both rapid and long-term effects on the vasculature including the local production of coagulation and fibrinolytic factors, modulation of extracellular matrix components, stimulation of antioxidants and the production of other vasoactive molecules, such as nitric oxide and prostaglandins, all of which are known to influence the development of vascular disease.

17β-estradiol manifests its physiological activities by activating two major classes of receptors, estrogen receptor α (ERα) and estrogen receptor β (ERβ). Some studies suggest that the beneficial effects of estradiol on ischemia/reperfusion injury result from the activation of ERβ (Gabel et al, *J Mol Cell Cardiol*, 38:289-297, 2005). Additionally, estrogen receptors
are reportedly found in two cellular compartments, the nucleus and the plasma membrane. Estradiol action on nuclear receptors is often referred to as “genomic action” involving activation of gene expression over a period of hours, days, weeks, or longer, while action on plasma membrane receptors is known as “non-genomic” involving activation of tyrosine kinase or MAP kinase signaling pathways over a period of minutes to hours (Chen et al, *J Clin Invest*, 103:401-406, 1999; Mendelsohn and Karas, *N Engl J Med*, 340:1801-1811, 1999). Genomic actions of estradiol are thought to include regulation of genes that effect vascular tone and atherosclerosis, induction of estrogen receptor expression, enhancement of endothelial cell growth and differentiation, and induction of nitric oxide synthase. Rapid, non-genomic actions of estradiol include rapid release of nitric oxide, vasodilation, and effects on endothelial and smooth muscle cells. Thus, for locally delivered estradiol, rapid release in addition to prolonged release of the drug may provide the most beneficial profile for amelioration of AMI or stabilization and healing of atherosclerotic lesions, such as thin-capped, or vulnerable, plaques.

**Mobilization of Cardiac Stem Cells**

Cardiac stem cells have recently been reported as existing in a variety of animal systems. Without being bound by any particular theory, it is believed that these cells do not effectively serve to regenerate the ischemically damaged myocardium that is the hallmark of AMI. However, it is thought that cardiac stem cells or other types of cells, such as early committed cells, do have the capacity to either regenerate damaged cardiac tissue or to enhance the function of existing cardiac tissue after AMI. Such cells are known to respond to stimulatory signals, such as peptide growth factors, which may attract them to the location of damaged tissue and/or induce them to differentiate into functional mycardiocytes. Certain PMAMI, such as IGF-1, HGF, CRF-1 and CRF-2 would be anticipated to act via inducing the mobilization and/or differentiation of cardiac stem cells, early committed cells, or other beneficial cells at the site of infarction. When these PMAMI are delivered locally to the site of infarction by the methods described herein, they mobilize endogenous stem cells or other beneficial cells to migrate to the site of infarction, where they either regenerate functional cardiac tissue or enhance the function of existing cardiac tissue. In the present invention, such PMAMI are formulated or encapsulated in microparticles containing GAGs. GAGs released
from cardiotherapeutic microparticles are believed to heal the damaged glycocalyx, while the released PMAM1 are believed to mobilize endogenous stem cells, early committed cells, or other beneficial cells. Both of these activities enhance the functional characteristics of ischemically damaged myocardium or scar tissue resulting from AMI.

Cardiac Disorders

Vulnerable plaque (VP) refers to a subgroup of high-risk coronary arterial plaques that are prone to rupture. VP rupture is often followed by thrombosis and occlusion of the coronary artery, which ultimately leads to the often fatal acute coronary syndrome (ACS) as well as to AMI. These plaques are not easily detected angiographically, perhaps because vessel occlusion is not manifest prior to plaque rupture. Vulnerable plaques demonstrate the following characteristics: a thin, fibrous cap (<65 μm), a lipid-rich and/or necrotic core, and increased macrophage activity. Awareness of VP has increased in recent years, with significant effort being devoted to development of detection methods such as intravascular ultrasound (IVUS), optical coherence tomography, intravascular magnetic resonance imaging, coronary spectroscopy, and intracoronary thermography (MacNeill et al, *Arterioscler Thromb Vasc Biol*, 23:1333-1342, 2003; El-Shafei and Kern, *J Invas Card*, 14:129-137, 2002). In addition, efforts are ongoing to establish the presence of soluble factors in blood (serum markers) that would serve to identify the existence of VP which in turn predict the onset of plaque rupture. Several serum markers have been proposed to serve this purpose: troponin-T, C-reactive protein, oxidized LDL, and soluble CD40 ligand, soluble ICAM-1, E-selectin, and soluble LOX-1. However, identification and diagnosis of VP has proven to be elusive, and the prospect of invasive diagnosis and treatment entails risk to the patient while proving burdensome both logistically and economically. Thus, the local / regional introduction of cardiotherapeutic microparticles into the coronary arteries provides many benefits: all plaque including VP receive the therapy regardless of the ability to visualize or locate them. In one embodiment treatment is administered during the course of a PTCA procedure. The dose for local treatment is notably less than that for systemic dosing.

For VP treatment, cardiotherapeutic microparticles offer the benefits of formulation with GAGs, which serve to target the microparticles to regions of damaged glycocalyx as well
as to potentially replace missing glycocalyx components, as well as delivery of a PMVP.
Some examples of PMVP are 17β-estradiol, an inhibitor of matrix metalloproteinases such as
MMP-1/collagenase-1, MMP-8/collagenase-2 or MMP-13/collagenase-3, an anti-
inflammatory (e.g. dexamethasone, FK506, pimecrolimus, sirolimus, everolimus, biolimus
A9, ABT578 or AP23573), a nitric oxide donor (e.g., nicorandil), an enhancer of
endothelilization (e.g. ghrelin), an IL-18 inhibitor (Maffia et al, Circulation, 114:430-437,
2006), inhibitors of the lysosomal proteinase cathepsin S (Rogers et al, Arterioscler. Thromb.
Vasc. Biol., 26:851-856, 2006), inhibitors of the cysteine proteinase legumain
angiogenesis (i.e. the vasa vasorum of VP) such as angiostatin, endostatin, SU5416, ZD6474,
VEGF inhibitors (e.g. bevacizumab) or 2-methoxyestradiol. For purposes of treatment with
cardiotherapeutic microparticles, in a particular embodiment therapeutic molecules are
delivered such that all regions of the coronary arteries containing VP receive an effective
amount. Certain PMAMI and PMVP, notably estradiol, are particularly useful as treatments
for both AMI and VP. Thus, in particular embodiments a single introduction of
cardiotherapeutic microparticles reaches the local and regional targets for both VP and AMI
when introduced into the coronary arteries.

The introduction of cardiotherapeutic microparticles upstream of a region that is
known or suspected to harbor vulnerable plaques is anticipated. In such a scenario, the
glycosaminoglycan portion of the formulation is believed to to cause some microparticles to
collect or congregate in the region of plaques. This is potentially related to the lack of a
healthy endothelium – resulting in a damaged glycocalyx layer – providing a surface that
tends to attract or bind glycosaminoglycans. Microparticles which collect in the region of
plaques provide glycosaminoglycan polymers in addition to localized micro-depots of
PMAMI.

Targeted Coatings for Therapeutic Microparticles

PMAMI- and PMVP-containing cardiotherapeutic microparticles may also be
encapsulated in other types of coatings, specifically a coating which additionally targets the
microparticle to the damaged tissue, i.e. the region of ischemic damage in the heart or areas of thin-capped atheromas (vulnerable plaques) in coronary arteries. The propensity of GAGs to localize to these damaged areas provides a first order degree of tissue targeting, without additional coatings. However, a second degree of tissue targeting for cardiotherapeutic microparticles may be achieved with additional coatings containing antibodies or other molecules that recognize specific molecular markers found in damaged areas. Alternatively, targeting antibodies, fragments thereof, or other targeting molecules may be incorporated in the microparticles themselves. The use of antibodies incorporated into the biocompatible coating of an implanted medical device is reported in U.S. Patent Application 20030229393.

In the practice of the present invention antibody coatings may be used on the surface of microparticles. Antibodies or portions thereof may be mixed with one of many possible biodegradable polymer matrices, such as dextran, polyglycolic acid (PGLA), polylactic acid (PLA), PGLA-PLA copolymers, polysaccharides, and/or phospholipids. Glycosaminoglycans, whether the same or similar as those used in the microparticle itself, are usefully used in the antibody-containing coating. N-acetylglucosamine (GlcNAc) conjugates (e.g. chitobiose or chitopentaose polymers) specifically bind to putative glycoside-binding proteins in infarcted myocardium (Ise et al, American Heart Association Scientific Sessions, Dallas, TX, 2005, Abstract #1010). In another embodiment, GlcNAc conjugates may be produced from GAGs themselves. Coatings are applied to microparticles in such a way that the antigen-binding or other targeting domains remain intact and able to bind to their target site. Potential target molecules in damaged tissue of the heart include but are not limited to matrix metalloproteinase-1, matrix metalloproteinase-8, matrix metalloproteinase-13, fibronectin, osteopontin, TLR4, IL1RL1-b, and Glycoside-binding Protein. Additional potential molecular targets may be identified using methods that are well known in the art, such as differential expression of mRNA and/or proteins in normal vs. diseased tissues. These methods have also been used to identify target molecules in rupture-prone arteries, containing vulnerable plaque. Examples of such targets include the adhesion molecule JAM-A and the metalloproteinase ADAMDEC1 (Papaspyridinos et al, Arterioscler. Thromb. Vasc. Biol., 26:1837-1844, 2006).
Potential target molecules in damaged coronary arteries containing vulnerable plaque are typically exposed on the surface of the VP, either (a) on the surface of cells, such as smooth muscle cells and macrophages, or (b) as part of the extracellular matrix that forms the fibrous cap of VP.


Examples of extracellular matrix markers that would allow targeting of the fibrous cap of VP are fibrillar collagen types I and III, specifically cleaved interstitial collagen (Horton et al, Ann NY Acad Sci, 947:329-336, 2001); metalloproteinases that specifically target collagen, thus rendering the fibrous cap to be thinner and more prone to rupture, such as MMP-1, MMP-8, MMP-13 (Horton et al, Ann NY Acad Sci, 947:329-336, 2001), and ADAMDEC1. Antibodies (monoclonal, polyclonal, humanized, and/or antibody fragments) are readily purchased or produced that recognize such molecular targets.

Administration of Cardiotherapeutic Microparticles

For PMAMI contemplated routes of administration are both systemic (whether oral or parenteral) delivered locally to the site of action. Local delivery of PMAMI offers specific
advantages. First, the coronary artery provides blood directly and specifically to the site of damage. Second, a effective amount is much reduced for local administration because the target tissue for PMAMI is typically less than 1% of the body mass served by systemic drug delivery. Third, a large number of patients suffering from AMI undergo PTCA (often including stent implantation) which provides a unique opportunity to deliver PMAMI to a particular coronary artery in an efficient and cost effective manner. Finally, the dosage and duration of treatment of PMAMI are closely controlled by local delivered to the coronary artery.

Local delivery of PMAMI is achieved in a specific embodiment by direct injection via catheter into the coronary artery lumen during the PTCA procedure. In a noted embodiment, PMAMI are formulated or encapsulated in degradable cardiotherapeutic microparticles consisting of glycosaminoglycan polymers, called cardiotherapeutic microparticles. Alternatively, cardiotherapeutic microparticles are delivered from coatings on indwelling devices, such as stents, such as stents implanted during PTCA, and elute PMAMI and glycosaminoglycans into the coronary artery lumen and hence directly into the infarcted zone. Cardiotherapeutic microparticles are also delivered via other devices such as drug-delivery balloons into the wall or lumen of the coronary artery. Finally, cardiotherapeutic microparticles are usefully delivered via direct injection into the infarcted region of the cardiac wall from an intramyocardial injection catheter (Boston Scientific Corporation Stiletto™ endocardial direct injection catheter system; Marshall et al, Molecular Therapy, 1:423-429, 2000; Karmarkar et al, Magnetic Resonance in Medicine, 51:1163-1172, 2004) or via direct injection into the wall of a diseased artery or vessel by infusion catheter (Mercator Medsystems MicroSyringe Infusion Catheter™, San Leandro, CA ).

The cardiotherapeutic microparticles used for treating a cardiac disorder are locally administered by any appropriate method. Methods for administration include, for example, intravenous or intra-arterial injection, a bolus injection via a catheter during PCTA, a coated or impregnated implantable device such as a stent, and injection directly into the target tissue, i.e. in and around the infarcted region of the heart. FIGURE 1 is a schematic showing one example of administration by intra-arterial injection. Cardiotherapeutic microparticles (2) are injected through catheter (4) into coronary artery (6) or directly into an infarcted region of the
cardiac wall (8). In some instances, Cardiotherapeutic microparticles (10) are eluted from a stent lodging in a vessel wall upstream of an infarcted region of cardiac wall (8). The formulated treatment is introduced by any convenient route (e.g., injection through a catheter into the juncture of the main coronary arteries and the aorta or delivered from a stent or balloon catheter to the site of VP). Targeted sites for PMVP therapy include the entire coronary artery tree, which is generally accessible at the juncture of the major coronary arteries with the aorta.


Delivery of cardiotherapeutic microparticles upstream of the site of AMI, from which point they would be carried by the flow of blood to the site of AMI, is usefully accomplished through the use of standard interventional devices including, but not limited to, drug-eluting stents, drug-delivery balloons, or PTCA catheters. In each case, an effective amount of cardiotherapeutic microparticles is ultimately delivered into the lumen of the coronary artery whose occlusion resulted in hypoxia and infarction. Cardiotherapeutic microparticles may be formulated in a variety of conventional pharmaceutical carriers including saline, emulsifiers, or other pharmaceutical excipients. Additionally, the cardiotherapeutic microparticles may be coated on a coronary stent either alone or embedded in polymers or other natural or synthetic carriers, for controlled elution into the lumen of the coronary artery. Typically cardiotherapeutic microparticles are taken via the coronary arterial system into the region of the heart that was subject to damage by ischemia. Typically further, the same coronary artery lesion that is undergoing treatment by PTCA and/or stenting will have been the cause of the arterial blockage that would lead to AMI due to ischemic and/or reperfusion injury. Delivery of cardiotherapeutic microparticles into the arterial lumen at the site of intervention will provide treatment to the precise site of coronary damage. If the treatment with
cardiotherapeutic microparticles is given independently of a PTCA procedure, delivery is still usefully made into the artery or arteries that are known to feed an affected region of the heart.

In one embodiment, cardiotherapeutic microparticles are delivered via one or both of two methods during the same interventional procedure, typically but not limited to PTCA. For example, a first dose is administered via catheter-based injection (formulated in one or more ways, such as in saline, in a pharmaceutical excipient, or additionally encapsulated with polymers) directly into the coronary arterial lumen, in the region of PTCA and stent placement, using the balloon catheter itself or a separate catheter (Guzman et al, Circulation, 94:1441-1448, 1996). The second administration of the same or a different PMAMI and/or cardiotherapeutic microparticles is via a coating on the coronary stent. Optionally, at least one therapeutic compound is embedded in a degradable or non-degradable polymer or natural coating, or alternatively formulated as cardiotherapeutic microparticles which are themselves embedded in a polymeric or other type of coating, and subsequently these coatings are applied to the surface of the coronary stent. PMAMI elute from the coating into the lumen of the coronary artery either by diffusion from the polymeric coating or by degradation of the coating, thus releasing the cardiotherapeutic microparticles.

For local delivery by drug delivery balloons, the cardiotherapeutic microparticles in a liquid formulation are usefully loaded into devices such as a triple-lumen balloon catheter (Infiltrator, manufactured by InterVentional Technology; Kaul et al, Circulation, 107:2551-2554, 2003), or a channel balloon catheter (Boston Scientific). From these devices microparticles are injected into the arterial wall, and thence released into the arterial lumen, from where they flow into the coronary infarcted zone. For local delivery directly into the target tissue, e.g., the coronary ventricular wall in and around the infarcted zone, cardiotherapeutic microparticles in liquid suspension/formulation are injected from an intramyocardial injection catheter (Boston Scientific Corporation Stiletto™ endocardial direct injection catheter system; Marshall et al, Molecular Therapy, 1:423-429, 2000; Karmarkar et al, Magnetic Resonance in Medicine, 51:1163-1172, 2004).

Many, if not all, situations in which a blocked artery is re-opened via PTCA result in some level of reperfusion injury. It is believed that introduction of cardiotherapeutic
microparticles enhances the repopulation of such injured regions with beneficial cells, thus reducing the extent or duration of injury.

A recently proposed clinical practice in interventional cardiology is termed “postconditioning,” whereby repetitive occlusion and reperfusion (using the PTCA balloon) immediately after revascularization serves to mitigate the deleterious effects of reperfusion, e.g. myocardial infarction size (Vinten-Johansen et al, *Circulation*, 112:2085-2088, 2005; Staas et al, *Circulation*, 112:2143-2148, 2005). One embodiment of the inventions disclosed herein encompasses the delivery of cardiotherapeutic microparticles for AMI that are specifically designed (based on timing and dose) to coincide with the postconditioning procedure. Thus, for each of several post-revascularization occlusions and reperfusion (typically four repetitions of occlusion and reperfusion, each lasting one minute, begun one minute after the initial reperfusion), a measured dose of cardiotherapeutic microparticles would be injected into the arterial lumen. This practice would impart greater ability to control dose, to improve selective targeting, and/or modulate duration of action for the cardiotherapeutic microparticles containing PMAMI.

**Pharmaceutical Formulations**

The cardiotherapeutic microparticles, or PMAMI/GAG microparticles, may be formulated in a variety of ways depending upon the intended route of administration. For direct intravenous or intra-arterial injection, standard injectable carriers known in the art are useful. Formulations include aqueous solutions including, for example, normal saline, Ringers, and 5% dextrose solutions. Pharmaceutical compositions according to the present invention may also comprise binding agents, filling agents, lubricating agents, disintegrating agents, suspending agents, preservatives, buffers, wetting agents, and other excipients. Examples of filling agents are lactose monohydrate, lactose hydrous, and various starches; examples of binding agents are various celluloses, preferably low-substituted hydroxylpropyl cellulose, and cross-linked polyvinylpyrrolidone; an example of a disintegrating agent is croscarmellose sodium; and examples of lubricating agents are talc, magnesium stearate, stearic acid, and silica gel. Examples of suspending agents are hydroxypropyl cellulose, methyl cellulose, hydroxyethyl cellulose, carboxymethyl cellulose sodium, hydroxypropyl
methylcellulose, acacia, alginic acid, carrageenin, and other hydrocolloids. Examples of preservatives, which control microbial contamination, are potassium sorbate, methylparaben, propylparaben, benzoic acid and its salts, other esters of parahydroxybenzoic acid such as butylparaben, alcohols such as ethyl or benzyl alcohol, phenolic compounds such as phenol, or quaternary compounds such as benzalkonium chloride.

For local injection via catheter at the site of PTCA, into the coronary artery lumen, similar formulations as above may be used. In addition, cardiotherapeutic microparticles may be additionally encapsulated with other polymers or coatings such that the particle diameter remains typically between 100nm and 2000nm. Such coatings could serve to delay release of the molecules into the surrounding serum or tissue, or to enhance the cardiotherapeutic microparticles being taken up by cells in the region of the target tissue, thus improving the efficiency of delivery and/or prolonging the availability of the PMAMI and/or GAG polymers. Examples of materials used to form or coat microparticles or nanoparticles are poly lactic acid, poly(D,L-lactide-co-glycolide) (PLGA), liposomes, and dextran (Jiang et al, Adv Drug Deliv Rev, 57:391-410, 2005; Bala et al, Crit Rev Ther Drug Carrier Syst, 21:387-422; Kayser et al, Curr Pharm Biotechnol; 6:3-5, 2005; U.S. Patent 6,805,879; U.S. Patent Application 20040191325).

For local delivery from stents or other implanted devices from which the flow of blood would carry eluted substances downstream to the target tissue, cardiotherapeutic microparticles can be mixed with polymers (co-dissolved or emulsified) and coated on such devices by spraying or dipping. The polymer is typically either bioabsorbable or biostable. A bioabsorbable polymer breaks down in the body and is not present sufficiently long after implantation to cause an adverse local response. Bioabsorbable polymers are gradually absorbed or eliminated by the body by hydrolysis, metabolic process, bulk erosion, or surface erosion. Examples of bioabsorbable materials include but are not limited to polycaprolactone (PCL), poly-D, L-lactic acid (DL-PLA), poly-L-lactic acid (L-PLA), poly(lactide-co-glycolide), poly(hydroxybutyrate), poly(hydroxybutyrate-covalerate), polydioxanone, polyorthoester, polyanhydride, poly(glycolic acid), poly(glycolic acid-cotrimethylene carbonate), polyphosphoester, polyphosphoester urethane, poly (amino acids), cyanoacrylates, poly(trimethylene carbonate), poly(iminocarbonate), copoly(etheresters), polyalkylene
oxalates, polyphosphaenes, polyiminocarbonates, and aliphatic polycarbonates. Biomolecules such as heparin, fibrin, fibrinogen, cellulose, starch, and collagen are typically also suitable. Examples of biostable polymers include parylene, polyurethane, polyethylene, polyethylene terephthalate, ethylene vinyl acetate, silicone and polyethylene oxide (PEO).

Cardiotherapeutic microparticles may also be coated on stents or other devices on which grooves, holes, micropores or nanopores have been engineered into the surface, such that the formulation to be delivered is sequestered within the pores and released slowly into the lumen of the coronary artery after the device has been implanted.

EXAMPLE 1
Treatment of AMI in a Porcine Model

Female or castrated male juvenile hybrid farm swine, 10-16 weeks old and weighing 35 +/- 10 kg, are utilized. Fasting is conducted prior to induction of anesthesia for device deployment, sample collection for serum chemistry and necropsy. Food, but not water, is withheld the morning of the procedure. To prevent or reduce the occurrence of thrombotic events, anti-platelet pharmacological therapy consisting of clopidogrel (75 mg per os [PO]) and acetylsalicylic acid (ASA; 325 mg, PO) is administered daily, with the exception of the implantation day, beginning at least 3 days prior to the scheduled procedure date.

Animals are tranquilized using intramuscular ketamine, azaperone or acepromazine and atropine. Anesthesia induction is achieved with propofol injected intravenously [IV] through a catheter in a peripheral ear vein. Upon induction of light anesthesia, the subject animal is intubated and supported with mechanical ventilation. Isoflurane (1 to 5.0% to effect by inhalation) in oxygen will be administered to maintain a surgical plane of anesthesia. Prophylactic antibiotic duplocillin LA® 0.05 ml/kg is given intramuscular [IM]. Intravenous fluid therapy is initiated and maintained throughout the procedure with saline (1 ml/kg/hour). The rate may be increased to replace blood loss or low systemic blood pressure.

The animal is placed in dorsal recumbency, and hair removed from access areas. Animals are kept warm throughout the preparation and the procedure. Limb-leads are placed, and electrocardiography established. The access site is prepared with chlorexidine, 70% isopropyl alcohol and providone, and the area is appropriately draped to maintain a sterile
field. After animal preparation, the femoral artery is accessed using a percutaneous approach. Alternatively, an incision will be made in the inguinal region to expose the femoral artery. An infiltration of bupivacain 0.5% (5 ml IM) on the femoral access site is performed to achieve local anesthesia and manage pain after surgery. A 7F or 8F-introducer arterial sheath is introduced and advanced into the artery. The sheath is connected to a pressure transducer for monitoring arterial pressure. An initial bolus of heparin (400 U/kg IV) is given and ACT performed approximately 5 minutes later. If ACT is under 300 seconds, an additional 100 to 400 U/kg of heparin is given. ACT is tested approximately every 20 minutes.

Under fluoroscopic guidance, a 7F guide catheter is inserted through the sheath and advanced to the appropriate location. After placement of the guide catheter, angiographic images of the coronary vessels are obtained with contrast media to identify the proper location for the deployment site. Quantitative angiography will be performed after injection of nitroglycerin 500 µg intracoronary [IC] to determine the appropriate vessel size for implantation and/or occlusion.

After visualization of the coronary artery anatomy, a segment of artery ranging from 2.6 mm to 3.5 mm mid-segment diameter is chosen, and a 0.014" guidewire will be inserted into the chosen artery. QCA is performed to accurately document the reference diameter for balloon angioplasty and/or stent placement.

Each stent delivery system or balloon catheter is prepared by applying vacuum to the balloon port; contrast/flush solution (50:50) is then introduced by releasing the vacuum. The appropriately sized balloon will be introduced into the appropriate artery by advancing the balloon catheter through the guide catheter and over the guidewire to the deployment site. The balloon is then inflated at a steady rate to a pressure sufficient to target a balloon:artery ratio of 1:1:1 (acceptable range of about 1.05:1 to about 1.15:1) and held for 60 minutes. A contrast injection is performed during full inflation to demonstrate occlusion with the balloon. During occlusion, monitoring is performed for heart functions, and other monitoring parameters include isoflurane level, SaO₂, pulse rate, blood pressure, temperature, O₂ flow, and tidal volume. After the occlusion period, vacuum is applied to the inflation device in order to deflate the balloon. Complete balloon deflation is verified with fluoroscopy.
Immediately upon reperfusion, the test treatment is initiated. In one embodiment, a coated stent containing a cardiotherapeutic microparticle is placed (target balloon:artery of about 1.15:1) in the same artery and region as the occlusion balloon. In a second embodiment, the cardiotherapeutic microparticle is administered through the balloon catheter (or other injection catheter) into the region of the balloon occlusion. In a third embodiment, a drug delivery balloon is utilized to introduce the cardiotherapeutic microparticle into the region of the balloon occlusion. A fourth embodiment administers a bolus injection via catheter and simultaneously provides the same, similar, or complementary PMAMI in a stent coating, which elutes over a period of hours, days, or weeks. In a fifth embodiment, four repetitions of occlusion and reperfusion, each lasting one minute, are begun one minute after the initial reperfusion (termed postconditioning). During each of these five reperusions, a measured dose (e.g., equivalent to 20% of a single dose) of cardiotherapeutic microparticles is introduced into the arterial lumen.

Following angiography, all catheters and sheaths are removed. If percutaneous access is achieved, pressure is applied to the access site until hemostasis is obtained. If a cutdown is performed, the femoral artery is ligated. The incision is closed in layers with appropriate suture materials.

The animals are placed in a pen and monitored during recovery from anesthesia for four to five hours following the procedure. Medical treatment including analgesia will be given as needed. Animals in apparent severe pain or distress, as determined by clinical observation and consultation with the facility veterinarian will be euthanized. ASA (325 mg/day PO) and clopidogrel (75 mg/day PO) are administered for the duration of the study. Moribidity/mortality checks and clinical observations are performed twice daily.

At the designated endpoint, typically 6 weeks post procedure, the animals are analyzed by MRI for LV function. Animals are then euthanized, the heart is excised, and the atria and great vessels are trimmed away. Next, the RV free wall is trimmed away from the LV (with septum intact). The LV is blotted dry, weighed and indexed by body weight (in kg). The LV is sectioned transversely into five equal segments from apex to base, immersed in 10%
buffered formalin, dehydrated at room temperature through ethanol series, and embedded in paraffin.

Serial 5-μm sections are prepared using a standard microtome. Sections are mounted and stained with hematoxylin and eosin (or trichrome) for determination of infarct size.

Quantitative histological analyses are performed, and infarct size is determined (Pfeffer et al, Circulation, 81:1161–1172, 1990). Infarct length is measured along the endo- and epicardial surfaces from each of the five LV segments (three sections per segment). Total LV circumference is measured along the endo- and epicardial surfaces from each of the five LV segments (three sections per segment). Infarct size is determined as percentage of total LV circumference. The ratio of scar length to body weight is calculated to exclude the potential influence of differences in body weight on infarct size.

Remodeling parameters. The maximum longitudinal dimension is measured before left ventricular sectioning. The LV is sectioned transversely into five equal segments from apex to base, and the maximum short-axis dimension after sectioning is measured. Outlines of the section rings and infarct scars are made on plastic overlays. "Thinning" ratio (ratio of average thickness of infarcted wall to average thickness of the normal wall) is measured by computerized planimetry. The maximum depth of infarct scar bulge in millimeters is measured on the contoured sections as an index of regional dilation. Bulging normalized to body weight is calculated. Ventricular volumes are computed from the short-axis areas and the long-axis length by the modified Simpson's rule, as used for echocardiographic studies during remodeling (Jugdutt et al, Circulation, 89:2297-2307, 1994).

The remaining sections are stained with Sirius red F3BA (0.1% solution in saturated aqueous picric acid) to discriminate between cardiomyocytes and collagen matrix (Wollert et al, Circulation, 95:1910–1917, 1997). Volume collagen fraction is calculated as the sum of all connective tissue areas divided by the total area of the image.

In the practice of this invention, therapeutically effective amounts of cardiotherapeutic microparticles are administered. In particular embodiments administration is via intra-arterial injection, intravenous injection, PTCA catheter, PTCA balloon, or indwelling device such as a stent. It is further contemplated that cardiotherapeutic
microparticles are administered by release from a coating on a coronary stent and additionally being injected through a PTCA catheter. Thus, in one embodiment, the cardiotherapeutic microparticles are delivered both immediately by injection and over a prolonged period by elution from a stent. Generally, the local drug delivery devices of this invention contain between about 0.001 mg and about 10.0 mg of cardiotherapeutic microparticles. Alternatively, intra-arterial or intravenous injection typically deliver between about 0.001 mg and about 100 mg of cardiotherapeutic microparticles. The exact dosage varies by disease severity, route of administration and the particular PMAMI used.

Nanoparticle and Microparticle Formulation

The therapeutic agents disclosed herein may be formulated along with pharmaceutically acceptable carriers and/or polymers, e.g. polyglycolic acid (PGLA), polylactic acid (PLA), PGLA-PLA copolymers, polysaccharides, glucosaminoglycans, and/or phospholipids, as spherical particles with diameters of about 100 nm to about 2,000 nm. Such particles are referred to commonly and interchangeably as either microparticles and/or nanoparticles. Many particles in this size range have the capability of entering living cells, and thus delivering the formulated therapeutic agent into cells of the target tissue. In such a system, delivery of the therapeutic agent is controlled in several ways: by the elution of the therapeutic agent via diffusion from the particle into the blood; by sequestration and subsequent release of the particles containing therapeutic agents in cells and extracellular matrix upstream of and in the target tissue; and by release of the therapeutic agent via biological breakdown, or degradation, of the particle itself. Details of such processes are described below ("Device Coatings").

Microparticles containing therapeutic agents may be produced by a variety of methods known in the art (Lemke and Hernandez-Trejo, Curr Pharm Biotechnol, 6:3-5, 2005), e.g. the emulsion-solvent evaporation technique (Sengupta et al, Nature, 436:568-572, 2005) or the stable aqueous/aqueous emulsion system (U.S. Patent 6,805,879). Different techniques are chosen based on the chemical, electrical, and hydrophobic properties of a given therapeutic agent. Microparticles are administered by themselves suspended in an appropriate solvent BUFFER system (Jiang et al, Adv Drug Deliv Rev, 10:391-410, 2005), by intra-arterial
injection (Guzman et al, *Circulation*, 94:1441-1448, 1996), by PTCA balloon delivery (Kaul et al, *Circulation*, 107:2551-2554, 2003), or any suitable method. Alternatively, microparticles are incorporated into a device coating such as those described herein. Microparticles themselves may incorporate more than one layer, with each layer possessing unique characteristics with regard to formulation and delivery of therapeutic agents.

**Glycosaminoglycan Microparticle Formulation**

Particular cardiotherapeutic microparticles disclosed herein consist of a therapeutic agent (PMAMI, pharmaceutical modulator of vulnerable plaque, or other therapeutic molecule) that is formulated as a microparticle with polymers that belong to a family, including glycosaminoglycans, that are capable of replacing, repairing or otherwise enhancing the damaged glycocalyx, consisting of the heparin sulfate proteoglycan (HSPG) layer and other glycoproteins, proteoglycans and glycosaminoglycans. Glycosaminoglycans (GAGs) are characterized as long unbranched polysaccharides made from a repeating disaccharide unit containing (1) either of the modified sugars N-acetylgalactosamine or N-acetylgalactosamine and (2) a uronic acid such as glucuronate or iduronate. Examples of GAGs are hyaluronic acid, dermatan sulfate, chondroitin sulfate, heparin, heparan sulfate, keratin sulfate, and synthetic analogs such as dextran sulfate.

In one embodiment, prior to production of microparticles, the GAG is combined with: (1) a PMAMI (e.g. proteins such as IGF-1, HGF, CRF-1, CRF-2, ghrelin, a compound that blocks platelet-endothelial cell adhesion molecules such as PECAM-1, IL-6/IL-6R or IL-6, relaxin, or clusterin, adiponectin, or small molecules like 17β-estradiol; a prostaglandin EP₃ receptor agonist, a caspase inhibitor, a potassium channel opener, a nitric oxide donor such as nicorandil, an aldosterone receptor antagonist such as spironolactone or eplerenone, a GP130 agonist, an IL-18 antagonist, a plasminogen activator inhibitor-1 antagonist, or a p38 MAP kinase inhibitor such as SB203580); or (2) a pharmaceutical modulator of vulnerable plaque (PMVP) such as 17β-estradiol, an inhibitor of matrix metalloproteinases such as MMP-1/collagenase-1, MMP-8/collagenase-2 or MMP-13/collagenase-3, an anti-inflammatory (e.g. dexamethasone, sirolimus, FK506, pimecrolimus, everolimus, biolimus A9, ABT578 or AP23573), a nitric oxide donor (e.g., nicorandil), an enhancer of endothelialization (e.g.
ghrelin), or inhibitors of angiogenesis (i.e. the *vasa vasorum* of VP) such as angiotatin, endostatin, SU5416, ZD6474, VEGF inhibitors (e.g. bevacizumab), a Lp-PLA$_2$ inhibitor (e.g. darapladib) or 2-methoxyestradiol.

The therapeutic agents (PMAMI or PMVP) used in accordance with the principles of this disclosure are usefully formulated as microparticles by suitable methods known in the art. For example and as discussed herein, the reactants and reaction conditions used to generate the polymer compositions disclosed herein may be modified to alter the properties of the final polymer composition. In some instances, properties such as the diffusion coefficients (e.g., the rate at which the therapeutic agents are able to diffuse through the polymer matrix), the rate of degradation of one or more of the polymer components, and the rate of the release of the therapeutic agents are manipulated by altering the reaction conditions and reagents, and hence the final polymer properties, used to generate the microparticles.

Bioabsorbable (biodegradable) polymers may be used in glycosaminoglycan microparticles, as coatings, inner layers, or cores, either combined with or separate from a therapeutic agent. Examples of bioabsorbable polymers are polyglycolic acid (PGLA), polylactic acid (PLA), PGLA-PLA copolymers, polysaccharides, and phospholipids. In addition, microparticles consisting of a therapeutic agent in a bioabsorbable polymer matrix may be coated with GAGs, thus enabling the microparticles to be targeted to the site of injury by virtue of the GAGs attraction to damaged glycoalyx, while the inner core would serve to deliver the therapeutic agent in a controlled manner.

Delivery of therapeutic agents from microparticles occurs via diffusion from the surface and/or interior of the particle into cellular cytoplasm, surrounding tissue, interstitial space, or vascular lumen. Additionally, *in vivo* hydrolytic degradation of the polymers making up the microparticle is a mechanism for release of the therapeutic agent, whereby metabolism of the polymers by endogenous enzymes may also play a role (Meyers et al., *J. Med. Chem.* 2000, 43, 4319-4327). Important factors influencing hydrolytic degradation include water permeability, chemical structure, molecular weight, morphology, glass transition temperature, additives, and other environmental factors such as pH, ionic-strength, site of release or sequestration, etc. The duration of sustained delivery can be adjusted from
few days up to one year by a person of ordinary skill in the art through proper selection of polymer, coating, and fabrication method.

One method of modulating the properties of the microparticles is to control the diffusion coefficient of one or more polymer layers. The diffusion coefficient relates to the rate at which a compound diffuses through a matrix. Methods for determining diffusion coefficients are described, for example, in U.S. Patent Nos. 5,786,439 and 5,777,060.

In another embodiment, linking agents are used to encapsulate and/or link the therapeutic agent to the polymer and/or GAG matrix or link the various components of the polymer matrix together (e.g., the different polymers that comprise the various coating layers; the bioactive agents in the polymer matrices etc.). Such linking agents include, for example, polyester amide (PEA), polyethylene imine (PEI), avidin-biotin complexes, photolinking and functionalized liposomes.

In another embodiment, therapeutic agents are modified by chemically linking them to a high molecular weight, water-soluble polymer carrier or GAG-carrier. This modified therapeutic agent is termed herein an agent-polymer conjugate. The agent conjugate is synthesized such that the chemical linkage of the agent to the water-soluble polymer can be manipulated to hydrolytically degrade, thereby releasing biologically active agent into the environment in which they are placed.

The agent-polymer conjugate is incorporated into a microparticle, formulated from a second biocompatible polymer. When administered into a tissue such as the arterial lumen, the controlled-release matrix releases the agent-polymer conjugate which further releases free agent molecules (PMAMI or PMVP) to treat the tissue in the immediate vicinity of the microparticles. The agent-polymer conjugates also diffuse within the tissue. As the agent conjugates diffuse, in blood or tissue, the bond between the polymer and the agent degrades in a controlled pattern, releasing the active agent.

There are several other variables that are controlled to produce a final product that is best suited for treating a certain disease with specific kinds of agents. A first variable is the size and characteristics of the polymer carrier. Either synthetic or naturally occurring
polymers may be used. While not limited to this group, some types of useful polymers include are polysaccharides (e.g., dextran and ficoll), proteins (e.g., poly-lysine), poly(ethylene glycol), and poly(methacrylates). Different polymers produce different diffusion characteristics in the target tissue or organ as a result of their different size and shape.

The rate of hydrolytic degradation, and thus of agent release, may be altered from minutes to months by altering the physico-chemical properties of the bonds between the agents and the polymer. While not wishing to be limited to the following types of bonds, artisans can bond therapeutic agents to water-soluble polymers using covalent bonds, such as ester, amide, amidoester, and urethane bonds. Ionic conjugates are also used. By changing the nature of the chemical association between water-soluble polymer and agent, the half-life of carrier-agent association is varied. This half-life of the agent-polymer conjugate in the environment in which it is placed determines the rate of active agent release from the polymer and, therefore, the degree of penetration that the agent-polymer conjugate can achieve in the target tissue. Other suitable hydrolytically labile bonds which can be used to link the agent to the water soluble polymer include thioester, acid anhydride, carbamide, carbonate, semicarbazone, hydrazone, oxime, iminocarbonate, phosphoester, phophazene, and anhydride bonds.

The rate of release is also affected by (a) stereochemical control (varying amounts of steric hindrance around the hydrolyzable bonds); (b) electronic control (varying electron donating/accepting groups around the reactive bond, controlling reactivity by induction/resonance); (c) varying the hydrophilicity/hydrophobicity of any optional spacer groups between the therapeutic agent and the polymer; (d) varying the length of the optional spacer groups (increasing length making the bond to be hydrolyzed more accessible to water); and (e) using bonds susceptible to cleavage by soluble blood plasma enzymes.

2444, 1991; J. Appl. Polymer Sci., 48: 1493-1500, 1992; Sherwood, et al., BioTechnology, 10: 1446-1449, 1992). Among the variables which affect conjugate release kinetics are: controlled release polymer composition, mass fraction of agent-polymer conjugate within the matrix (increasing mass fraction increases release rate), particle size of agent-polymer conjugate within the matrix (increasing particle size increases release rate), composition of polymeric agent conjugate particles, and polymer size (increasing surface area increases the release rate), and polymer shape of the controlled release matrix. Suitable polymer components for use as controlled-release matrices include poly(ethylene-co-vinyl acetate), poly(DL-lactide), polyglycolide, copolymers of lactide and glycolide, and polyanhydride copolymers.

As discussed in U.S. Pat. No. 6,300,458, hydroxypolycarbonates (HPC) are used as hydroxyl functional polymers that bind therapeutic agents or carbohydrate polymers chemically or via hydrogen bonding. These copolymers have properties attractive to the biomedical area as is or by conversion to the HPC product provided by hydrolysis or by in vivo enzymatic attack. A feature of these polymers is their tendency to undergo surface erosion. Heterogeneous hydrolysis theoretically would better preserve the mechanical strength and physical integrity of the matrix during biodegradation, which is highly desirable in terms of predictable performance. To maximize control over the release process, it is desirable to have a polymeric system which degrades from the surface and deters the permeation of the agent molecules. Achieving such a heterogeneous degradation requires the rate of hydrolytic degradation on the surface to be much faster than the rate of water penetration into the bulk.

As noted above, the polymer compositions disclosed herein allow for the controlled release of therapeutic agents. This controlled release is modulated by the pH of the environment in which the polymer compositions function. In this context, one embodiment includes the controlled release of the therapeutic agents from a hydrophobic, pH-sensitive polymer matrix (see, for example, U.S. Patent No. 6,306,422). A polymer of hydrophobic and weakly acidic comonomers is used in the controlled release system. Weakly basic comonomers are used and the active agent is released as the pH drops. For example, a pH-sensitive polymer releases the therapeutic agents when exposed to a higher pH environment as
the polymer gel swells. Such release can be made slow enough so that the therapeutic agent remains at significant levels for a clinically useful period of time.

Related embodiments provide additional compositions for releasing therapeutic microparticles using a dual phase polymeric agent-delivery composition. These dual phase polymeric compositions comprise a continuous biocompatible gel phase, and a discontinuous particulate phase comprising defined GAG microparticles containing the therapeutic agents to be delivered (see, for example, U.S. Patent No. 6,287,588). Typically in such embodiments, a cardiotherapeutic microparticle is entrained within a biocompatible polymeric gel matrix. The therapeutic agent release is contained in the GAG microparticle phase alone or in both the microparticles and the gel matrix. The release of the therapeutic agent is prolonged over a period of time, and the delivery is modulated and/or controlled. In addition, the second agent is loaded in the same or different GAG microparticles and/or in the gel matrix. Alternatively, layered GAG microparticles may be produced in which, for example, the inner core consists of a particular polymer carrying the therapeutic agent while an outer layer consisting of the same or a different material may either carry the therapeutic agent and release it with different release kinetics (Sengupta et al, Nature, 436:568-572, 2005), or not carry the therapeutic agent and serve to control its release from the inner core. The outer layer may also serve to target the microparticles containing the therapeutic agent to specific tissue locations, by virtue of the composition of this layer, e.g. containing GAGs that naturally bind to regions of damaged glycocalyx, or of specialized elements within the layer such as antibodies or fragments thereof that bind to molecules that are preferentially found in damaged tissue in the region of AMI, VP, or other coronary syndromes.

This invention also encompasses drug-eluting devices, whereby GAG microparticles entrained in the coating of a device, such as a vascular stent, are released into the vascular lumen. These microparticles are released at a constant rate or at a multi-phasic rate. For example, in one embodiment, the release comprises an initial burst (immediate release) of the GAG microparticles present at or near the surface of the coating layer, a second phase during which the release rate is slower or sometimes no microparticle is released, and a third phase during which most of the remainder of the therapeutic microparticles are released as erosion proceeds.
EXAMPLE 2
Preparation of Cardiotherapeutic Microparticles

Therapeutic microparticles containing PMAMI or PMVP are prepared using GAG. In the first embodiment, hyaluronic acid (sodium hyaluronate) is used to prepare microparticles (S.J. Kim et al, Journal of Controlled Release, 104:323-335, 2005).

\[
\begin{align*}
\text{Sodium hyaluronate} & \quad \text{(HA)}
\end{align*}
\]

(see exemplary structure, above) is a naturally occurring glycosaminoglycan. It is a major constituent of the extracellular matrix of connective tissue present in almost all vertebrates, and can be found at high concentrations in the umbilical cord, in the aqueous and vitreous humor of the eye, in synovial joint fluid, in some strains of bacteria, and in chicken combs.

In its natural form, HA typically exists as a sodium salt (sodium hyaluronate), which can form a highly viscous fluid (viscoelastic) with exceptional lubricating qualities. It plays an important role in a number of physiological functions including cell protection and lubrication, maintenance of the structural integrity of tissues, transport of molecules, and fluid retention and regulation. It is biocompatible and bioabsorbable.

Sodium hyaluronate (m.w. 2000 to 40,000) is obtained from Genzyme Biosurgery (Cambridge, MA). The following equipment is used: stirred cell (Amicon, Beverly, MA), tip sonicator (Vibracell, Sonics and Materials, Newtown, CT), laboratory-scale spray dryer (B-191, Buchi Labortechnik AG, Switzerland), pilot scale spray dryer, vacuum drying oven, particle size analyzer (Master sizer MS20, Malvern, Worcestershire, England).

Preparation of prototype formulation of PMAMI or PMVP in HA. Sodium hyaluronate (HA) is dissolved in water using a mechanical stirrer. The bulk solution of PMAMI is buffer changed into 10 mM sodium phosphate at pH 7.4 by ultra-filtration using a stirred cell. In one embodiment, the PMAMI is a mixture of two peptide hormones, IGF-1
and HGF, dissolved in 10 mM sodium phosphate at pH 7.4, at e.g. between 0.1 mg/ml and 50 mg/ml. The buffer-changed PMAMI solution is sterile-filtered through a 0.2μm filter, and then mixed with HA solution using a mechanical stirrer for 30 minutes. The ratio of PMAMI to HA is 1:1. After the addition of Tween 80 at a concentration of 0.01%, the solution is spray-dried to prepare a first core particle using a laboratory scale mini spray drier. Lecithin is separately solubilized in ethanol using a magnetic stirrer. An equal amount of the first core particle to lecithin is dispersed in the lecithin solution. The resulting mixture with a mass ratio of PMAMI:HA:lecithin :: 1:1:2 is spray-dried to produce the final second microparticles coated with lecithin using the minispray dryer.

In another embodiment, the PMAMI or PMVP is e.g. 17β-estradiol in the form of a microcrystalline solid. The PMAMI/PMVP solid powder is mixed with the HA solution at a concentration between 0.01 mg/ml and 50 mg/ml to form a suspension which is subsequently formed into microparticles by spray drying or other methods.

EXAMPLE 3

Preparation of PMAMI or PMVP in dextran. Dextran (mw 100,000 to 1,000,000) is dissolved in water at a concentration of 10 to 50 w/v % (solution A). A second aqueous solution (solution B) is prepared with polyethylene glycol (PEG, mw 1,000 to 12,000) with the PEG concentration ranging from 10 to 40 w/v %. PMAMI (e.g. peptide hormones) is added into 0.25 ml solution A, at a concentration between, e.g., 0.1 mg/ml and 50 mg/ml, and mixed with pipeting. The resulting solution is dispersed slowly into solution B under stirring at room temperature. The volume ratio of solution A and B is 1:1. This results in a stable aqueous-aqueous emulsion system, with the PMAMI-containing dextran being the dispersed phase and the PEG being the continuous phase.

In another embodiment, 17β-estradiol in the form of a microcrystalline solid is mixed with solution A, e.g. at a concentration between 0.01 mg/ml and 50 mg/ml, prior to mixing with solution B.

Microparticles of dextran containing PMAMI or PMVP are prepared by freeze-drying the stable emulsions. Most of the dextran particles are dispersed in a solid matrix formed by the continuous phase, PEG. The PEG is removed by washing the lyophilized powder with methylene chloride or acetonitrile. These solvents do not swell or dissolve the dried dextran
phase. After freeze-drying, the diameter of the dispersed phase is between 0.1 to 3 μm. Alternative methods utilize glycosaminoglycans in place of dextran such as dextran sulfate, hyaluronic acid, dermatan sulfate, chondroitin sulfate, heparin, heparan sulfate, or keratin sulfate. This preparation method is further exemplified in Patent No. US 6,803,879.

The microparticles of PMAMI containing dextran or a glycosaminoglycan may be further encapsulated into a matrix of polylactic / polyglycolic acid (PLGA). This is done by a solid-in-oil-in-water emulsification process. PLGA (mw 50,000 - 100,000) with a lactic to glycolic ratio of 50:50 and 75:25 is used. Microparticles prepared previously (above) are suspended in a PLGA / dichloromethane solution (10 to 20 %), then added into a water solution containing 0.1 to 10% sodium chloride and 0.5 to 5% polyvinyl alcohol (PVA) or PEG or polyvinyl pyrrolidone (PVP) under stirring. The volume ratio of the two solutions was 1:5. After an emulsion was formed, the organic solvent was extracted by pouring the system into large volume of cold water (10 times of the emulsion) under stirring. Before solvent extraction, the PLGA droplets are transparent, within which the encapsulated microparticles are evenly distributed. After hardening by the removal of solvents, the PLGA particles lose transparency.

EXAMPLE 4

Antibody-targeted cardiotherapeutic microparticles. Microparticles containing glycosaminoglycans, dextran, PLA, PLGA, or other biodegradable polymers may also be coated with an antibody-containing targeting coating, or the antibody-containing biocompatible polymer matrix may be incorporated in the microparticle itself.

As used herein, the term "antibody" refers to one type of monoclonal, polyclonal, humanized, or chimeric antibody or a combination thereof, wherein the monoclonal, polyclonal, humanized or chimeric antibody binds to one antigen or a functional equivalent of that antigen. The term antibody fragment encompasses any fragment of an antibody such as Fab, F(ab').sub.2, and can be of any size, i.e., large or small molecules, which have the same results or effects as the antibody. (An antibody encompasses a plurality of individual antibody molecules equal to 6.022.times.10.sup.23 molecules per mole of antibody).

Monoclonal antibodies useful in the method of the invention may be produced according to the standard techniques of Kohler and Milstein (Continuous cultures of fused
cells secreting antibody of predefined specificity. Nature 265:495-497, 1975, incorporated herein by reference), or can be obtained from commercial sources. Antigens derived from vulnerable plaque or regions of myocardial infarction can be used as the immunogen to produce monoclonal antibodies directed against the target tissue. Alternatively, differential expression of mRNA or protein can be used to compare diseased tissue with normal tissue, thus identifying appropriate antigens to be used to raise antibodies that specifically bind to diseased tissue.

Antibodies that recognize and bind to regions of vulnerable plaque and/or myocardial infarction are incorporated into the matrix of the cardiotherapeutic microparticle or into a surface layer of same, either covalently or noncovalently. This method has been well documented and used clinically (U.S. Patent Application 2003/0229393; Aoki et al, J Am Coll Cardiol, 45:1574-1579, 2005). In one embodiment antibodies may be incorporated into the matrix by mixing the antibodies with the matrix solution and then producing microparticles as described above, or using other known methods. In another embodiment, whole antibodies with or without antibody fragments are covalently coupled to the matrix. For instance, the antibodies are tethered covalently to the matrix through the use of hetero- or homobifunctional linker molecules. As used herein the term "tethered" refers to a covalent coupling of the antibody to the matrix by a linker molecule. The use of linker molecules in connection with the present invention typically involves covalently coupling the linker molecules to the matrix after it is formed into microparticles. After covalent coupling to the matrix, the linker molecules provide the matrix with a number of functionally active groups that can be used to covalently couple one or more types of antibody. The linker molecules may be coupled to the matrix directly (i.e., through the carboxyl groups), or through well-known coupling chemistries, such as, esterification, amidation, and acylation. The linker molecule may be a di- or tri-amine functional compound that is coupled to the matrix through the direct formation of amide bonds, and provides amine-functional groups that are available for reaction with the antibodies. For example, the linker molecule could be a polyamine functional polymer such as polyethyleneimine (PEI), polyallylamine (PALLA) or polyethyleneglycol (PEG). A variety of PEG derivatives, e.g., mPEG-succinimidyl propionate or mPEG-N-hydroxysuccinimide, together with protocols for covalent coupling, are commercially available from Shearwater Corporation, Birmingham, Ala. (See also, Weiner et
al., Influence of a poly-ethyleneglycol spacer on antigen capture by immobilized antibodies. J. Biochem. Biophys. Methods 45:211-219 (2000), incorporated herein by reference). It will be appreciated that the selection of the particular coupling agent may depend on the type of antibody used and that such selection may be made without undue experimentation. Mixtures of these polymers can also be used. These molecules contain a plurality of pendant amine-functional groups that can be used to surface-immobilize one or more antibodies.

In another embodiment, microparticles containing PMAMI / PMVP may be produced from conventional polymers such as PLGA, and coated afterwards with GAGs alone or GAGs containing antibodies that would serve to target the microparticle to regions of cardiovascular damage. The microparticles are formulated utilizing an emulsification solvent evaporation technique. In brief, 600 mg of PLGA is dissolved in 24 mL of methylene chloride. The organic phase is emulsified into 120 mL of an aqueous phase containing 2.5% polyvinyl alcohol (30 to 70 K molecular weight) by sonication over an ice bath for 10 minutes with the use of a probe-type sonicator with an energy output set at 55 W to form an oil-in-water emulsion. The oil-in-water emulsion thus formed is further stirred at room temperature over a magnetic stir plate for 18 hours to evaporate the organic solvents. Microparticles formed in this fashion are recovered by ultracentrifugation at 145 000g, washed three times with water to remove polyvinyl alcohol, resuspended, and lyophilized. For entrainment of PMAMI / PMVP (between 1 mg and 1000 mg) was dissolved in the initial step into the organic phase, followed by the same sequence as above. In one embodiment, 17β-estradiol is dissolved in 4 mL acetone and PLGA (600 mg) in 24 mL methylene chloride. Both solutions are mixed to form an organic phase. The subsequent steps are the same as described previously for microparticle formulation. Microparticles are characterized for particle size with the use of a laser defractometer (NICOMP, model 370). The particle size ranges from 90 to 250 nm, with approximately 75% of the particles in the range of 100 to 200 nm, with a mean size of between 100 to 200 nm. Scanning electron microscopy also demonstrates uniform particle size distribution. Such uniform microparticles can then be coated using known methods (Sengupta et al, Nature, 436:568-572, 2005) with antibodies that are formulated with a GAG polymer (as described above), or alternatively with a matrix consisting exclusively of a GAG polymer.
All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.
What is claimed is:

CLAIMS

1. A method for treating a cardiac disorder in a patient, said method comprising locally administering a cardiotherapeutic microparticle consisting of a glycosaminoglycan matrix containing a therapeutic molecule selected from the group consisting of an estrogen, a prostaglandin EP₃ receptor agonist, a caspase inhibitor, a potassium channel opener, a nitric oxide donor, an aldosterone receptor antagonist, an inhibitor of platelet-endothelial cell adhesion molecules, IL-6/sIL-6R, IL-6, a GP130 agonist, an IL-18 antagonist, a glycosaminoglycan analog, a plasminogen activator inhibitor-1 antagonist, relaxin, clusterin, adiponectin, p38 MAP kinase inhibitor, a cardiac regeneration factor, insulin-like growth factor 1 (IGF-1), hepatocyte growth factor (HGF), erythropoietin (EPO), carbamylated EPO (CEPO), and/or tissue-protective cytokines (TCPs).

2. The method of claim 1, wherein said cardiac disorder is selected from the group consisting of acute myocardial infarction, a chronic ischemic condition, reperfusion injury, chronic heart disease, vulnerable plaques, and cardiac fibrosis.

3. The method of claim 1, wherein said cardiac disorder is acute myocardial infarction.

4. The method of claim 1, wherein said microparticles are administered by intravenous or intra-arterial injection.

5. The method of claim 1, wherein said microparticles are administered during percutaneous transluminal coronary angioplasty.
6. The method of claim 5, wherein said microparticles are administered using a drug delivery percutaneous transluminal coronary angioplasty balloon.

7. The method of claim 1, wherein said microparticles are administered using an implantable intra-arterial stent.

8. The method of claim 1, wherein said microparticles display on their surface antibodies, antibody fragments or peptides that recognize molecular markers selected from the group consisting of MMP-1, MMP-8, MMP-13, fibronectin, osteopontin, TLR4, and IL1RL1-b.

9. An implantable device comprising a cardiotherapeutic microparticle consisting of a glycosaminoglycan matrix containing a therapeutic molecule selected from the group consisting of a prostaglandin EP3 receptor agonist, a caspase inhibitor, a potassium channel opener, a nitric oxide donor, an aldosterone receptor antagonist, an inhibitor of platelet-endothelial cell adhesion molecules, IL-6/sIL-6R, IL-6, a GP130 agonist, an IL-18 antagonist, a glycosaminoglycan analog, a plasminogen activator inhibitor-1 antagonist, relaxin, clusterin, adiponectin, p38 MAP kinase inhibitor, a cardiac regeneration factor, insulin-like growth factor 1 (IGF-1), hepatocyte growth factor (HGF), erythropoietin (EPO), carbamylated EPO (CEPO), and tissue-protective cytokines (TCPs).

10. The device of claim 9, wherein said microparticle is coated on said device.

11. The device of claim 9, wherein said microparticle is contained within a polymer coating on said device.

12. The device of claim 9, wherein said device is a stent.
13. A method for treating a cardiac disorder in a patient, said method comprising locally administering a cardiotherapeutic microparticle consisting of a glycosaminoglycan matrix containing a therapeutic molecule selected from the group consisting of an estradiol, an inhibitor of MMP-1/collagenase-1, MMP-8/collagenase-2 or MMP-13/collagenase-3, dexamethasone, FK506, pimecrolimus, sirolimus, everolimus, biolimus A9, ABT578, AP23573, nicorandil, an IL-18 antagonist, a VEGF inhibitor, a legumain inhibitor, a Lp-PLA₂ inhibitor, a cathepsin S inhibitor, ghrelin, angiotatin, endostatin, SU5416, ZD6474, and 2-methoxyestradiol.

14. The method of claim 13, wherein said cardiac disorder is vulnerable plaque.

15. The method of claim 13, wherein said microparticles display on their surface antibodies, antibody fragments, or peptides that recognize molecular markers selected from the group consisting of CD40 ligand, lectinlike oxidized low-density lipoprotein receptor-1 (LOX-1), pregnancy-associated plasma protein A (PAPP-A), monomeric (or modified) C-reactive protein (mCRP), fibrillar collagen types I and III, specifically cleaved interstitial collagen, MMP-1, MMP-8, MMP-13, α,β₃ integrin, ADAMDEC1, JAM-A, RAGE, and vascular cell adhesion molecule-1 (VCAM-1).

16. An implantable device comprising a cardiotherapeutic microparticle consisting of a glycosaminoglycan matrix containing a therapeutic molecule selected from the group consisting of an estradiol, an inhibitor of MMP-1/collagenase-1, MMP-8/collagenase-2 or MMP-13/collagenase-3, dexamethasone, FK506, pimecrolimus, sirolimus, everolimus, biolimus A9, ABT578, AP23573, nicorandil, an IL-18 antagonist, a VEGF inhibitor, a legumain inhibitor, a Lp-PLA₂ inhibitor, a cathepsin S inhibitor, ghrelin, angiotatin, endostatin, SU5416, ZD6474, and 2-methoxyestradiol.

17. The device of claim 16, wherein said microparticle is coated on said device.
18. The device of claim 16, wherein said microparticle is contained within a polymer coating on said device.

19. The device of claim 16, wherein said device is a stent.