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[Continued on next page]

(54) Title: MYOCARDIAL TISSUE TARGETING PEPTIDES



Heart



Lung



Kidney



Spleen



Liver

Fig. 1

(57) Abstract: A myocardial tissue targeting peptide consisting of about 5 to about 25 amino acids, wherein at least a portion of the amino acid sequence of the peptide is homologous to at least 5 consecutive amino acids of SEQ ID NO:1.



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PATENT**MYOCARDIAL TISSUE TARGETING PEPTIDES****RELATED APPLICATION**

[0001] This application claims priority from U.S. Provisional Application No. 61/429,941, filed January 5, 2011, the subject matter, which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] This application relates to myocardial tissue targeting peptides, compositions and methods of delivering therapeutic and/or imaging agents to the myocardium of a subject, and to methods of treating a cardiomyopathy in a subject.

BACKGROUND

[0003] Ischemia is a condition wherein the blood flow is completely obstructed or considerably reduced in localized parts of the body, resulting in anoxia, reduced supply of substrates and accumulation of metabolites. Although the extent of ischemia depends on the acuteness of vascular obstruction, its duration, tissue sensitivity to it, and developmental extent of collateral vessels, dysfunction usually occurs in ischemic organs or tissues, and prolonged ischemia results in atrophy, denaturation, apoptosis, and necrosis of affected tissues.

[0004] In ischemic cardiomyopathy, which are diseases that affect the coronary artery and cause myocardial ischemia, the extent of ischemic myocardial cell injury proceeds from reversible cell damage to irreversible cell damage with increasing time of the coronary artery obstruction.

[0005] Gene therapy has evolved as a viable therapeutic option for treating patients with ischemic conditions by delivering pro-angiogenic proteins to infarct zones, thus providing cardiac benefit. However, injecting plasmids into the myocardial wall requires an invasive procedure in order to deliver the therapeutic gene. Injecting the plasmid directly into the myocardium, ensures maximum efficacy of the therapeutic gene, as it is delivered into the micro environment associated with the damaged tissue, therefore, improving the chances of the gene being taken up by these cells. However, delivery of naked plasmid into the tissues, increases the probability of being subject to endosomal degradation. Therefore, only a smaller portion of the plasmid injected is taken up by the cells and then translated to

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the therapeutic protein. In order to protect the genes from degradation, scientists have studied various techniques to package the plasmid and safely deliver it to organs. Although this technique protects the DNA from being degraded, it does not eliminate the need for invasive surgery and there remains a need for improving penetration of the genes into the cells.

SUMMARY

[0006] This application relates to a myocardial tissue targeting peptide. The targeting peptide consists of about 5 to about 25 amino acids. At least a portion of the amino acid sequence of the peptide is homologous to at least 5 consecutive amino acids of SEQ ID NO:1. In some aspects, the peptide includes an amino acid sequence consisting of SEQ ID NO: 1. In some aspects the peptide includes an amino acid sequence consisting of SEQ ID NO: 2. In some aspects the myocardial tissue targeting peptide is selective for a weakened, ischemic, and/or peri-infarct region of mammalian myocardial tissue.

[0007] The application also relates to a method of delivering a therapeutic and/or imaging agent to myocardial tissue of a subject. The method includes administering to the subject a nanoparticle. The nanoparticle includes a polymeric carrier and an agent. In some aspects, the agent is encapsulated by the nanoparticle. The nanoparticle is conjugated to a myocardial tissue targeting peptide. The myocardial tissue targeting peptide consists of about 5 to about 25 amino acids. At least a portion of the amino acid sequence of the myocardial tissue targeting peptide is homologous to at least 5 consecutive amino acids of SEQ ID NO:1. In some aspects, the myocardial tissue targeting peptide includes an amino acid sequence consisting of SEQ ID NO: 1. In some aspects the myocardial tissue targeting peptide includes an amino acid sequence consisting of SEQ ID NO: 2.

[0008] In some aspects, the myocardial tissue targeting peptide is selective for a weakened, ischemic, and/or peri-infarct region of mammalian myocardial tissue. In some aspects, the nanoparticles can be administered systemically.

[0009] In some aspects the polymeric carrier can include PLGA. In some aspects, the therapeutic and/or imaging agent is encapsulated by the nanoparticles. In some aspects of the application, the therapeutic agent can include a cytokine, such as SDF-1. The therapeutic agent can also include a nucleic acid, such as an SDF-1 expressing plasmid DNA.

[0010] The application further relates to a method of treating a cardiomyopathy in a subject. The method includes administering systemically to a subject a therapeutically

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effective amount of nanoparticles. The nanoparticles include a polymeric carrier and SDF-1. The nanoparticle is conjugated to a myocardial tissue targeting peptide. The myocardial tissue targeting peptide consists of about 5 to about 25 amino acids. At least a portion of the amino acid sequence of the myocardial tissue targeting peptide is homologous to at least 5 consecutive amino acids of SEQ ID NO:1. The therapeutically effective amount of nanoparticles is the amount effective to cause functional improvement in at least one of the following parameters: left ventricular volume, left ventricular area, left ventricular dimension, cardiac function, 6-minute walk test, or New York Heart Association (NYHA) functional classification. In some aspects, the myocardial tissue targeting peptide consists of 7 amino acids. In some aspects the myocardial tissue targeting peptide consists of 9 amino acids.

[0011] In some aspects the myocardial tissue is selective for a weakened, ischemic, and/or peri-infarct region of mammalian myocardial tissue. In some aspects the polymeric carrier can include PLGA. In some aspects, the SDF-1 is encapsulated by the nanoparticles. In certain aspects, the SDF-1 includes an SDF-1 expressing plasmid DNA.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Fig. 1 illustrates specificity of peptide interaction with heart tissues. Rodent hearts were frozen and then stained for His antibody. A "His" tag was attached to the peptide sequence thus a positive His stain correlated with a positive peptide identification. Peptides were infused via the tail vein and translocated to the heart tissue specifically.

[0013] Fig. 2 illustrates the dose response with un-conjugated nanoparticles encapsulating 6C. Cardiac fibroblasts were cultured and nanoparticles encapsulating a fluorescent dye, 6C, were added to this in varying doses. The signal obtained correlates to the amount of 6C that released inside the cells. A 200µg dose was used for future studies.

[0014] Fig. 3 illustrates time response with un-conjugated nanoparticles encapsulating 6C. Rat cardiac fibroblasts were cultured onto 6 well plated and 200µg of nanoparticles without any targeting peptide were added to this. The time taken for release of the nanoparticles into the cells and the endocytosis was determined by imaging cells at various time points following the addition of nanoparticles. 6C signal was obtained 1 hour following the addition and lasted for more than 24 hours.

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[0015] Fig. 4 illustrates time response of nanoparticles with 6C conjugated with RR peptide. Rat cardiac fibroblasts were cultured onto 6 well plated and 200µg of nanoparticles with targeting peptide were added to this. On imaging these cells at various time points, 6C signal was obtained 1 hour following the addition and lasted for more than 24 hours similar to the results obtained from un-conjugated nanoparticles.

[0016] Fig. 5 illustrates *in vitro* HPLC analysis to determine amount of 6C in cardiac fibroblasts. Both conjugated and unconjugated nanoparticles had similar sizes. A quantitative analysis between the unconjugated and conjugated nanoparticles revealed similar amounts of nanoparticles being taken up by cardiac fibroblasts in culture. However, unsonicated nanoparticles that had a larger size exhibited lower uptake.

[0017] Fig. 6 illustrates *in vivo* HPLC analysis between infarct and healthy regions of the heart. On comparing infarcted tissue and healthy tissue for the amount of nanoparticles that released the fluorescent dye, 6C in them, we observed a greater amount of nanoparticles that targeted the infarct heart as compared to the healthy heart when a targeting peptide was used to conjugate the nanoparticles.

[0018] Fig. 7 illustrates the difference in uptake in infarcted tissue with conjugation of nanoparticles. A greater number of nanoparticles targeted the infarct heart when a targeting peptide was conjugated to the surface of the PLGA polymer. (n=5), p<0.05

DETAILED DESCRIPTION

[0019] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the application(s) belong. All patents, patent applications, published applications and publications, Genbank sequences, websites and other published materials referred to throughout the entire disclosure herein, unless noted otherwise, are incorporated by reference in their entirety. In the event that there are a plurality of definitions for terms herein, those in this section prevail. Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this application belongs. Commonly understood definitions of molecular biology terms can be found in, for example, Rieger et al., Glossary of Genetics: Classical and Molecular, 5th edition, Springer-Verlag: New York, 1991; and Lewin, Genes V, Oxford University Press: New York, 1994.

[0020] Methods involving conventional molecular biology techniques are described herein. Such techniques are generally known in the art and are described in detail in methodology treatises, such as *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989; and *Current Protocols in Molecular Biology*, ed. Ausubel et al., Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates). Methods for chemical synthesis of nucleic acids are discussed, for example, in Beaucage and Carruthers, *Tetrahedron Letts.* 22:1859-1862, 1981, and Matteucci et al., *J. Am. Chem. Soc.* 103:3185, 1981. Chemical synthesis of nucleic acids can be performed, for example, on commercial automated oligonucleotide synthesizers. Immunological methods (*e.g.*, preparation of antigen-specific antibodies, immunoprecipitation, and immunoblotting) are described, *e.g.*, in *Current Protocols in Immunology*, ed. Coligan et al., John Wiley & Sons, New York, 1991; and *Methods of Immunological Analysis*, ed. Masseyeff et al., John Wiley & Sons, New York, 1992. Conventional methods of gene transfer and gene therapy can also be adapted for use in the application. See, *e.g.*, *Gene Therapy: Principles and Applications*, ed. T. Blackenstein, Springer Verlag, 1999; *Gene Therapy Protocols (Methods in Molecular Medicine)*, ed. P. D. Robbins, Humana Press, 1997; and *Retro-vectors for Human Gene Therapy*, ed. C. P. Hodgson, Springer Verlag, 1996.

[0021] As used herein, "nucleic acid" refers to a polynucleotide containing at least two covalently linked nucleotide or nucleotide analog subunits. A nucleic acid can be a deoxyribonucleic acid (DNA), a ribonucleic acid (RNA), or an analog of DNA or RNA. Nucleotide analogs are commercially available and methods of preparing polynucleotides containing such nucleotide analogs are known (Lin et al. (1994) *Nucl. Acids Res.* 22:5220-5234; Jellinek et al. (1995) *Biochemistry* 34:11363-11372; Pagratis et al. (1997) *Nature Biotechnol.* 15:68-73). The nucleic acid can be single-stranded, double-stranded, or a mixture thereof. For purposes herein, unless specified otherwise, the nucleic acid is double-stranded, or it is apparent from the context.

[0022] As used herein, "DNA" is meant to include all types and sizes of DNA molecules including cDNA, plasmids and DNA including modified nucleotides and nucleotide analogs.

[0023] As used herein, "nucleotides" include nucleoside mono-, di-, and triphosphates. Nucleotides also include modified nucleotides, such as, but are not limited to, phosphorothioate nucleotides and deazapurine nucleotides and other nucleotide analogs.

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[0024] As used herein, the term "subject" or "patient" refers to animals into which the compositions described herein can be introduced. Included are higher organisms, such as mammals and birds, including humans, primates, rodents, cattle, pigs, rabbits, goats, sheep, mice, rats, guinea pigs, cats, dogs, horses, chicken and others.

[0025] As used herein the term "cardiomyopathy" refers to the deterioration of the function of the myocardium (*i.e.*, the actual heart muscle) for any reason. Subjects with cardiomyopathy are often at risk of arrhythmia, sudden cardiac death, or hospitalization or death due to heart failure.

[0026] As used herein, the term "ischemic cardiomyopathy" is a weakness in the muscle of the heart due to inadequate oxygen delivery to the myocardium with coronary artery disease being the most common cause.

[0027] As used herein the term "ischemic cardiac disease" refers to any condition in which heart muscle is damaged or works inefficiently because of an absence or relative deficiency of its blood supply; most often caused by atherosclerosis, it includes angina pectoris, acute myocardial infarction, chronic ischemic heart disease, and sudden death.

[0028] As used herein the term "myocardial infarction" refers to the damaging or death of an area of the heart muscle (myocardium) resulting from a blocked blood supply to that area.

[0029] As used herein the term "6-minute walk test" or "6MWT" refers to a test that measures the distance that a patient can quickly walk on a flat, hard surface in a period of 6 minutes (the 6MWD). It evaluates the global and integrated responses of all the systems involved during exercise, including the pulmonary and cardiovascular systems, systemic circulation, peripheral circulation, blood, neuromuscular units, and muscle metabolism. It does not provide specific information on the function of each of the different organs and systems involved in exercise or the mechanism of exercise limitation, as is possible with maximal cardiopulmonary exercise testing. The self-paced 6MWT assesses the submaximal level of functional capacity. (See for example, AM J Respir Crit Care Med, Vol. 166. Pp 111-117 (2002))

[0030] As used herein "New York Heart Association (NYHA) functional classification" refers to a classification for the extent of heart failure. It places patients in one of four categories based on how much they are limited during physical activity; the limitations/symptoms are in regards to normal breathing and varying degrees in shortness of breath and or angina pain:

NYHA Class	Symptoms
I	No symptoms and no limitation in ordinary physical activity, e.g. shortness of breath when walking, climbing stairs etc.
II	Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.
III	Marked limitation in activity due to symptoms, even during less-than-ordinary activity, <i>e.g.</i> , walking short distances (20–100 m). Comfortable only at rest.
IV	Severe limitations. Experiences symptoms even while at rest . Mostly bedbound patients.

[0031] Embodiments of this application relate to myocardial tissue targeting peptides that are selective for mammalian myocardial tissue. The myocardial tissue targeting peptide consists of about 5 to about 25 amino acids. At least a portion of the amino acid sequence of the myocardial tissue targeting peptide is homologous to at least 5 consecutive amino acids of SEQ ID NO:1. It was found that a 7 amino acid peptide ligand having the amino acid sequence RQPRMKR (SEQ ID NO:1) has a high affinity *in vivo* to weakened, ischemic, and/or peri-infarct regions of the heart compared to healthy heart and liver tissue when administered systemically to a mammalian subject.

[0032] In some embodiments, the myocardial tissue targeting peptide described herein includes an amino acid sequence of SEQ ID NO:1. In other embodiments, the myocardial tissue targeting peptide has an amino acid sequence that consists of SEQ ID NO:1. The myocardial tissue targeting peptide can be targeted to and selectively bind to myocardial tissue when systemically administered to a mammalian subject. The myocardial tissue targeting peptide can bind to the myocardial tissue with a higher affinity than non-myocardial tissues.

[0033] In some aspects, when systemically administered to a subject, the myocardial tissue targeting peptide can bind to myocardial tissue in the subject with at least 2-fold greater affinity (*e.g.*, at least 3-fold, at least 5-fold, at least 10-fold, or at least 25-fold greater

affinity) than to a non-myocardial tissue. Thus, a myocardial tissue targeting peptide can be selected based on binding kinetics to myocardial tissue.

[0034] In some embodiments the targeting peptide is flanked on either sides by cysteine residues to provide a 9 amino acid circular CRQPRMKRC ligand (SEQ ID NO:2) while retaining selective affinity for the myocardium. Additional amino acid residues can be added to both the N terminus and C terminus ends of the peptide as long as it does not substantially inhibit the selective affinity of the targeting peptide. Candidate myocardial targeting peptides can be screened *in vitro* by determining affinity to myocardial tissue (*e.g.*, cardiac fibroblasts) in, for example, a multi-well format. Candidate myocardial tissue targeting peptides also can be screened *in vivo* by assessing the rate and timing of excretion of candidate myocardial tissue targeting peptide from the body. In this respect, the myocardial tissue targeting peptide may be expelled from the body via the kidneys.

[0035] A myocardial tissue targeting peptide of the present invention can be synthesized using any well known method previously described. Following peptide synthesis, peptides can be analyzed to insure proper synthesis. For example, proper synthesis can be confirmed using mass spectrometry and/or purity of the peptides can be verified using reverse phase liquid chromatography. In an exemplary embodiment, the mass of the peptides is determined using a mass spectrometer and was recorded at 1794.5923 units using a 4700 Reflector Spectrometer.

[0036] Another aspect of the application relates to a method of delivering one or more active agents (*e.g.*, a therapeutic and/or a diagnostic agent) to myocardial tissue of a subject. It was found that the systemic delivery of agents contained within a polymeric carrier nanoparticle conjugated to a targeting peptide having SEQ ID NO:1 enhances the uptake of the agents into cells of the myocardial tissue. It is contemplated that encapsulating a therapeutic and/or diagnostic agent of interest into a nanoparticle (*e.g.*, a PLGA polymeric nanoparticle) tagged with a myocardial tissue targeting peptide can provide a targeting encapsulated active agent capable of homing to the myocardial tissue upon systemic administration to a subject. The targeting peptide, when conjugated to the polymeric complex can be able to direct the polymeric carrier nanoparticle composition to the injured myocardium. The polymer can then be able to release the encapsulated active agent(s) into the area of injury.

[0037] Thus, a nanoparticle described herein can be used in a method of delivering a therapeutic and/or diagnostic agent to myocardial tissue of a subject. In some embodiments the composition is administered systemically.

[0038] A nanoparticle composition described herein can include a polymeric carrier and a therapeutic and/or diagnostic agent. Nanoparticles can generally entrap or encapsulate the therapeutic and/or diagnostic agent in a stable and reproducible manner. The nanoparticle composition is conjugated to a myocardial tissue targeting peptide described herein consisting of about 5 to about 25 amino acids, wherein at least a portion of the amino acid sequence of the peptide is homologous to at least 5 consecutive amino acids of SEQ ID NO:1. In some embodiments, the myocardial tissue targeting peptide is selective for and targets a weakened, ischemic, and/or peri-infarct region of a subject's myocardial tissue.

[0039] In some aspects, the myocardial tissue targeting peptide conjugated to a nanoparticle can reversibly bind to myocardial tissue such that the myocardial targeting peptide is eventually released from myocardial tissue and expelled from the body.

[0040] The myocardial tissue targeting peptide can remain bound to myocardial tissue for a period of time effective to allow the conjugated nanoparticle to deliver the therapeutic and/or diagnostic agent(s) to the target cells (*e.g.*, myocardial cells). The myocardial tissue targeting peptide can remain bound to the myocardium for about 1 or more days (*e.g.*, about 2 days, about 3 days, or about 7 days) to about 1 year or more (*e.g.*, about 330 days, about 365 days, or about 400 days), after which the myocardial tissue targeting peptide is expelled from the body. The myocardial tissue targeting peptide can remain bound to myocardium for about 7 or more days (*e.g.*, about 7 days, about 14 days, or about 21 days) to about 6 months or more (*e.g.*, about 90 days, about 120 days, or about 150 days). For example, a myocardial tissue targeted nanoparticle can remain bound to the myocardium for 30 days, during which time the drug is released and the nanoparticle degrades. After about 45 days the myocardium targeting peptide would be released from the myocardium and eventually excreted, *e.g.*, after 30 or 45 days of treatment.

[0041] In some embodiments, the polymeric carrier nanoparticle includes a biodegradable polymer. By "biodegradable" is meant a compound that can be decomposed or degraded by biological or biochemical processes. The products of these biodegradable polymers may be completely broken down and removed from the body by normal metabolic pathways. Biodegradable polymers have advantages over other carrier systems in that they need not be

surgically removed when drug delivery is completed and that they can provide direct drug delivery to the systemic circulation.

[0042] In some embodiments, the nanoparticle can be a particle of approximately spherical shape measuring less than about 1000 nm in diameter. The size of the nanoparticle can affect various therapeutic properties of the nanoparticle, including the rate of decomposition, the rate of release of an active agent, the ability to access the myocardial tissue of a mammal, and the ability to avoid macrophages in the circulatory system. Furthermore, smaller nanoparticles are known to cross into the cellular matrix, typically by endocytosis, and the size requirements of the nanoparticles are an important characteristic in transportability. Nanoparticles may enter a cell via the cellular caveolae, typically 20-60 nm openings that participate in receptor-mediated uptake processes, and via receptor-mediated endocytosis in clathrin-coated pits, typically in the range of 150-200 nm.

[0043] In one embodiment, the nanoparticles can have a diameter of about 10 nm to about 1000 nm. In another embodiment, the nanoparticles can have a diameter of about 50 to about 500 nm, more preferably from about 100 to about 400 nm, and even more preferably from about 100 to about 250 nm. In an exemplary embodiment the average diameter of a conjugated nanoparticle is 103 ± 11 nm.

[0044] The size distribution of the nanoparticles can also be important since different sizes produce different release rates and different drug loading levels. The size range of the nanoparticles can be narrow, broad, or multimodal. The number of nanoparticles within a given size range can be greater than about 75%, greater than about 85%, greater than about 95%, or greater than about 99%. For example, if greater than 99% of the nanoparticles were within the range of 150-250 nm, then the distribution might be considered narrow, whereas greater than 75% of the nanoparticles within the range of 10-1000 nm might be considered broad. Alternatively, the size distribution of particles can be characterized by the relative polydispersity. Relative polydispersity can be determined by a Coulter Nanosizer, and indicates the relative distribution around the median diameter. A relative polydispersity of 1 indicates a monodisperse sample, while increasing values indicate a broader distribution within the sample. The relative polydispersity can be less than about 5, preferably less than about 3, and more preferably less than about 2.

[0045] A polymeric carrier nanoparticle can include any suitable biodegradable polymer, such as biodegradable polymers that are currently in use or are being developed for controlled

drug delivery *in vivo*. For example, the biodegradable polymer can be a polyester, a polylactone, a polycarbonate, a polyamide, or a polyol. The polyester can include of poly(lactic acid), commonly known as PLA, poly (glycolic acid), commonly known as PGA, and their copolymers, commonly known as poly(lactic-co-glycolic) acid or PLGA. The nanoparticles composed of PLGA can have any ratio of PLA and PGA, *e.g.*, a lactic acid: glycolic acid ratio (*e.g.*, molar ratio) of about 95:5 to about 5:95, preferably of about 75:25 to about 25:75, or more preferably of about 50:50. The PLGA copolymer can be a random copolymer or block copolymer of lactic acid and glycolic acid. The block copolymers can have 2, 3, 4, or more blocks of PLA and PGA. The lactic acid component can be racemic, enantomerically enriched with the D or the L isomer, or enantiopure.

[0046] The hydrolysis of polyesters, by both nonenzymatic and enzymatic esterase-based pathways, leads to glycolic and lactic acids which are easily metabolized in the citric acid cycle. While degradation by nonenzymatic processes has no dependency on the chirality of the polyesters, the rate of cleavage by biological esterase shows some dependency on the chirality of the lactic acid. Therefore, the enantiomeric ratio of the polyester could affect the degradation rate of the nanoparticle and by extension the release rate of the therapeutic agent, and provides for flexibility in controlling the rate of drug delivery.

[0047] By varying the monomer ratios in the polymer processing and by varying the processing conditions, the resulting polymer can exhibit drug release capabilities for months or even years. Increasing the ratio of PLA increases the relative hydrophobicity of the nanoparticle, while increasing the ratio of PGA increases the hydrophilicity. The resultant nanoparticle can therefore bind therapeutic and diagnostic agents with a wide range of hydrophobicities and hydrophilicities, and the subsequent release of these agents can be optimized by controlling the monomer ratios and processing conditions. In addition, crystallinity, molecular weight, and amounts of any residual solvents used in the preparation may also affect the release rates of active agents.

[0048] In another embodiment, a biodegradable nanoparticle can include poly(ethylene glycol) or poly(ethylene oxide), commonly known as PEG or PEO, which is a polyether formed either from ethylene glycol or ethylene oxide as a monomer. PEG can be incorporated into the nanoparticle by any suitable approach, *e.g.*, as a block copolymer of the biodegradable polymer graft or as an attachment (*e.g.*, covalent) to the nanoparticle or its surface, as a blend of PEG and the biodegradable polymer used during formation of the

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nanoparticle, or as a coating of the PEG onto the nanoparticle surface. The PEG can be associated with the polymer by ionic, covalent, coordinate, hydrogen bonding, van der Waals, and other intermolecular forces, or be a simple blend. When PLGA, PLA, or PGA and PEG are utilized, *e.g.*, as a triblock copolymer, the PEG or PEO often, though not necessarily, is the central block, and the polyester chains are at either end of the polymer. Studies have evaluated the effect of the length of a central PEG block (*J. Contrl. Rel.*, 24, 81 (1993)) as well as the length of outer PLA blocks (*Macromolecules*, 29, 50, 57 (1996)) on water absorption and degradation of these copolymers.

[0049] Nanoparticles having a PEG component may avoid detection and sequestration by the mononuclear phagocyte system and the reticuloendothelial system and subsequent elimination in the liver or kidneys. Accordingly, a PEG component in a nanoparticle may increase the residence time and the effectiveness of the nanoparticles in drug treatment. The use of PEG or PEO on proteins or nanoparticles has been shown to increase the circulating lifetime of these foreign species. Examination of the biodistribution of nanoparticles containing PEG shows greatly enhanced circulation times for PLGA-PEG nanoparticles over PLGA nanoparticles alone (Li et al., *J. Cntrl. Rei.*, 39, 315 (1996)). Specific cellular uptake studies have shown that a surface layer of PEG will avoid premature capture of nanoparticles by the mononuclear phagocyte system with PLGA-PEG copolymers, with PEG segments of 5,000 molecular weight showing the greatest protection (Jaeghere et al., *J. Drug Target.*, 8, 143 (2000)). The types of active agents recently successfully incorporated and released from PLGA-PEG copolymers include rhodamine B (Panoyan et al., *Proc. Inti. Sym. Cntrl. Rei. Bioact. Mat.*, 28, 5120 (2001)), taxol (for microparticle preparation) (Das et al., *J. Biomed. Mat. Res.*, 55, 96 (2001)), adriamycin (Lie et al. *J. App. Poly. Sci.*, 80 1976 (2001)), doxorubicin (Yoo et al., *J. Contrl. Rei.*, 70, 63 (2001)), and VEGF (for microsphere preparation) (King et al., *J. Biomed. Mat. Res.*, 51,383 (2000)).

[0050] The nanoparticles can be prepared in any manner depending on the nature of components to be included in the nanoparticle. For example, the preparation methods for biodegradable microparticles can be used to prepare the nanoparticles of the invention. Most preparations are based on solvent evaporation or extraction techniques (see, for example, D. H. Lewis "Controlled Release of Bioactive Agents from Lactide/Glycolide Polymers" in *Biodegradable Polymers as Drug Delivery Systems*, Marcel Dekker, p. 1 (1990)). The simplest methods involve dissolving the polymer in an appropriate organic solvent and

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suspending this solution in an aqueous continuous phase which contains an appropriate surfactant. Continuous stirring then allows for evaporation of the organic solvent and hardening of the microparticles. The key factors that control the size and size distribution of these particles are the polymer concentration in the solvent, the amount and type of surfactant, and the stirring rate. The solvents used in these techniques can include dichloromethane, acetone, methanol, ethyl acetate, acetonitrile, chloroform, and carbon tetrachloride.

[0051] In an exemplary embodiment, the peptides can be conjugated to the nanoparticles in a two step process. In the first step, the surfaces of the nanoparticles are activated by suspending the nanoparticles in borate buffer (50mM, pH 5.0) by sonication for 30 sec on an ice bath. This is followed by the addition of DENACOL (40 mg), an epoxy that helps conjugation of the peptide on the surface, and the catalyst zinc tetrahydrofluoroborate hydrate (50 mg), which are dissolved in an equal volume of buffer to the nanoparticle solution. This mixture can be stirred gently for example, for 30 minutes at 37°C.

Nanoparticles can then be separated by ultracentrifugation for example at 30000 rpm for 20 minutes at 4°C. Any unreacted DENACOL can be removed by multiple wash steps.

[0052] In the second step, the peptide can be conjugated to the surface of the activated nanoparticles by suspending the nanoparticles in borate buffer (4 ml) and stirring into a solution containing an amount of the targeting peptides in borate buffer. This reaction can be carried out, for example, for 2 hours at 37°C. The unreacted peptide can then be removed by ultracentrifugation and the final nanoparticles suspension can be lyophilized for 48 hours.

[0053] A therapeutic agent encapsulated by a nanoparticle can be any known compound or mixture. In some embodiments, the therapeutic agent includes one or more drugs, proteins, cytokines, nucleic acids, hormones, steroids, enzymes or mixtures thereof. Exemplary therapeutic agents include, but are not limited to, anti-inflammatory agents, diuretics, vasodilators, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor agonists, beta-blockers such as certain angiogenic growth factors, p38 MAP kinase inhibitors, pro-angiogenic compounds, such as angiopoietin-1, RhoA, Rac1, VEGF and bFGF, IGF-1, adeno-cyclin A2, erythropoietin, ATP-sensitive potassium channel openers, antineoplastic agents, hormones, analgesics, anesthetics, neuromuscular blockers, antimicrobials or antiparasitic agents, antiviral agents, interferons, antidiabetics, antihistamines, anticoagulants, and the like.

[0054] A nucleic acid therapeutic agent can include, but is not limited to, plasmid DNA encoding VEGF, bFGF, and/or SDF-1. In certain embodiments, the biologically active or therapeutic agent can include SDF-1 or a SDF-1 expressing plasmid DNA.

[0055] The nanoparticles can contain more than one therapeutic agent. For example, two therapeutic agents could have a synergistic effect when delivered simultaneously to the myocardial tissue, or a complementary effect. A nanoparticle can also be designed to deliver the two reagents at different points in time and/or at different rates. One drug could have a higher affinity for the nanoparticle, due to, for example, hydrophobicity/hydrophilicity, acidity/basicity, or favorable enantiomer-enantiomer interactions, and therefore a slower release rate than the complementary drug.

[0056] A diagnostic agent encapsulated by the nanoparticle can include any diagnostic agent used for *in vivo* cardiac contrast imaging such as myocardial contrast echocardiography (MCE) and other imaging modalities used to provide contrast in cardiac tissue such as computed tomography (CT), magnetic resonance imaging (MRI) coronary angiogram, electroanatomical mapping, fluoroscopy and single-photon emission computed tomography (SPECT). It will be appreciated that other imaging techniques that can define a weakened, ischemic, and/or peri-infarct region in a subject can also be used.

[0057] Exemplary diagnostic agents include rare earth metals such as manganese, ytterbium, gadolinium, europium, as well as irons, fluorophores (fluorescein, dansyl, quantum dots, and fluorocarbons).

[0058] Radionuclides are also useful both as diagnostic and therapeutic agents. Typical diagnostic radionuclides include ^{99m}Tc , ^{95}Tc , ^{111}In , ^{62}Cu , ^{64}Cu , ^{67}Ga and ^{68}Ga , and therapeutic nuclides include ^{186}Re , ^{188}Re , ^{153}Sm , ^{166}Ho , ^{177}Lu , ^{149}Pm , ^{90}Y , ^{212}Bi , ^{103}Pd , ^{109}Pd , ^{159}Gd , ^{140}La , ^{198}Au , ^{199}Au , ^{169}Yb , ^{175}Yb , ^{165}Dy , ^{166}Dy , ^{67}Cu , ^{105}Rh , ^{111}Ag , and ^{192}Ir . Means to attach various radioligands to the nanoparticles of the invention are understood in the art.

[0059] The therapeutic and/or diagnostic agent and polymeric carrier nanoparticle may be combined in a number of different ways depending upon the application of interest. For example, the therapeutic and/or diagnostic agent may be non-covalently associated with the nanoparticle, may be coupled to the nanoparticle or may be coupled to the nanoparticle through spacer moieties.

[0060] Nanoparticles loaded with a therapeutic and/or diagnostic agent conjugated to myocardial tissue targeting peptides can be administered systemically at a dose ranging from

10 µg of nanoparticles to 100 g of nanoparticles. The appropriate dose of the composition administered to a mammal in accordance with the inventive method should be sufficient to affect the desired response in the mammal over a reasonable time frame. Dosage will depend upon a variety of factors, including the age, species, and size of the mammal. Dosage also depends on the particular therapeutic agent, nanoparticle formulation, and myocardial tissue targeting peptide that are employed. The size of the dose also will be determined by the route, timing, and frequency of administration as well as the existence, nature, and extent of any adverse side effects that might accompany administration and the desired physiological effect. Some situations, such as exposure of a mammal to multiple rounds of chemotherapy or radiation therapy, may require prolonged treatment involving multiple administrations. The actual dose of the inventive composition can range from about 0.05 milligrams per kilogram of body mass to about 100 milligrams per kilogram of body mass.

[0061] This application further relates to methods of treating a cardiomyopathy in a subject, wherein the cardiomyopathy results in reduced and/or impaired myocardial function. It was previously found that functional improvement of ischemic myocardial tissue is dependent on the amount, dose, and/or delivery of SDF-1 administered to the ischemic myocardial tissue and that the amount, dose, and/or delivery of SDF-1 to the ischemic myocardial tissue can be optimized so that myocardial functional parameters, such as left ventricular volume, left ventricular area, left ventricular dimension, or cardiac function are substantially improved.

[0062] Therefore, a method of treating a cardiomyopathy in a subject is provided. A cardiomyopathy treated by the compositions and methods herein can include cardiomyopathies associated with a pulmonary embolus, a venous thrombosis, a myocardial infarction, a transient ischemic attack, a peripheral vascular disorder, atherosclerosis, ischemic cardiac disease and/or other myocardial injury or vascular disease. A method of treating the cardiomyopathy can include systemically administering to weakened myocardial tissue, ischemic myocardial tissue, and/or apoptotic myocardial tissue, such as the peri-infarct region of a heart following myocardial infarction, an amount of stromal-cell derived factor-1 (SDF-1) that is effective to cause functional improvement in at least one of the following parameters: left ventricular volume, left ventricular area, left ventricular dimension, cardiac function, 6-minute walk test (6MWT), or New York Heart Association (NYHA) functional classification.

[0063] In one example, SDF-1 loaded nanoparticles conjugated to a targeting peptide described herein can be administered systemically to a mammalian subject having a weakened region, an ischemic region, and/or peri-infarct region of myocardial tissue in which there is a deterioration or worsening of a functional parameter of the heart, such as left ventricular volume, left ventricular area, left ventricular dimension, or cardiac function as a result of an ischemic cardiomyopathy, such as a myocardial infarction. The deterioration or worsening of the functional parameter can include, for example, an increase in left ventricular end systolic volume, decrease in left ventricular ejection fraction, increase in wall motion score index, increase in left ventricular end diastolic length, increase in left ventricular end systolic length, increase in left ventricular end diastolic area (*e.g.*, mitral valve level and papillary muscle insertion level), increase in left ventricular end systolic area (*e.g.*, mitral valve level and papillary muscle insertion level), or increase in left ventricular end diastolic volume as measured using, for example, using echocardiography.

[0064] In some embodiments, the amount of SDF-1 loaded nanoparticles administered to subject having a weakened region, ischemic region, and/or peri-infarct region of myocardial tissue can be an amount effective to improve at least one functional parameter of the myocardium, such as a decrease in left ventricular end systolic volume, increase in left ventricular ejection fraction, decrease in wall motion score index, decrease in left ventricular end diastolic length, decrease in left ventricular end systolic length, decrease in left ventricular end diastolic area (*e.g.*, mitral valve level and papillary muscle insertion level), decrease in left ventricular end systolic area (*e.g.*, mitral valve level and papillary muscle insertion level), or decrease in left ventricular end diastolic volume measured using, for example, using echocardiography as well as improve the subject's 6-minute walk test (6MWT) or New York Heart Association (NYHA) functional classification.

[0065] In other embodiments of the application, the amount of SDF-1 loaded nanoparticles systemically administered to the subject with a cardiomyopathy is the amount effective to improve left ventricular end systolic volume in the mammal by at least about 10%, and more specifically at least about 15%, after 30 days following administration as measured by echocardiography. The percent improvement is relative to each subject treated and is based on the respective parameter measured prior to or at the time of therapeutic intervention or treatment.

[0066] In further embodiments of the application, the amount of SDF-1 loaded nanoparticles systemically administered to the subject with a cardiomyopathy is effective to improve left ventricular end systolic volume by at least about 10%, improve left ventricular ejection fraction by at least about 10%, and improve wall motion score index by about 5%, after 30 days following administration as measured by echocardiography.

[0067] In still further embodiments of the application, the amount of SDF-1 loaded nanoparticles systemically administered to the subject with a cardiomyopathy is the amount effective to improve vasculogenesis of the weakened region, ischemic region, and/or peri-infarct region by at least 20% based on vessel density or an increase in cardiac perfusion measured by SPECT imaging. A 20% improvement in vasculogenesis has been shown to be clinically significant (Losordo Circulation 2002; 105:2012).

[0068] In other embodiments of the application, the amount of loaded nanoparticles systemically administered to the subject with a cardiomyopathy is effective to improve six minute walk distance at least about 30 meters or improve NYHA class by at least 1 class.

[0069] The SDF-1 described herein can be systemically administered to the subject following tissue injury (*e.g.*, myocardial infarction) to about hours, days, weeks, months, or years after onset of down-regulation of SDF-1. The period of time that the SDF-1 is administered to the subject can comprise from about immediately after onset of the cardiomyopathy (*e.g.*, myocardial infarction) to about days, weeks, months or years after the onset of the ischemic disorder or tissue injury.

[0070] SDF-1 in accordance with the application that is loaded into and encapsulated by myocardial tissue targeting peptide conjugated nanoparticles and systemically administered to subject can have an amino acid sequence that is substantially similar to a native mammalian SDF-1 amino acid sequence. The amino acid sequence of a number of different mammalian SDF-1 protein are known including human, mouse, and rat. The human and rat SDF-1 amino acid sequences are at least about 92% identical (*e.g.*, about 97% identical). SDF-1 can comprise two isoforms, SDF-1 alpha and SDF-1 beta, both of which are referred to herein as SDF-1 unless identified otherwise.

[0071] The SDF-1 can have an amino acid sequence substantially identical to SEQ ID NO: 3. The SDF-1 can also have an amino acid sequence substantially similar to one of the foregoing mammalian SDF-1 proteins. For example, the SDF-1 can have an amino acid sequence substantially similar to SEQ ID NO: 4. SEQ ID NO: 4, which substantially

comprises SEQ ID NO: 3, is the amino acid sequence for human SDF-1 and is identified by GenBank Accession No. NP954637. The SDF-1 can also have an amino acid sequence that is substantially identical to SEQ ID NO: 5. SEQ ID NO: 5 includes the amino acid sequences for rat SDF and is identified by GenBank Accession No. AAF01066.

[0072] The SDF-1 in accordance with the application can also be a variant of mammalian SDF-1, such as a fragment, analog and derivative of mammalian SDF-1. Such variants include, for example, a polypeptide encoded by a naturally occurring allelic variant of native SDF-1 gene (*i.e.*, a naturally occurring nucleic acid that encodes a naturally occurring mammalian SDF-1 polypeptide), a polypeptide encoded by an alternative splice form of a native SDF-1 gene, a polypeptide encoded by a homolog or ortholog of a native SDF-1 gene, and a polypeptide encoded by a non-naturally occurring variant of a native SDF-1 gene.

[0073] SDF-1 variants have a peptide sequence that differs from a native SDF-1 polypeptide in one or more amino acids. The peptide sequence of such variants can feature a deletion, addition, or substitution of one or more amino acids of a SDF-1 variant. Amino acid insertions are preferably of about 1 to 4 contiguous amino acids, and deletions are preferably of about 1 to 10 contiguous amino acids. Variant SDF-1 polypeptides substantially maintain a native SDF-1 functional activity. Examples of SDF-1 polypeptide variants can be made by expressing nucleic acid molecules that feature silent or conservative changes. One example of an SDF-1 variant is listed in US Patent No. 7,405,195, which is herein incorporated by reference in its entirety.

[0074] SDF-1 polypeptide fragments corresponding to one or more particular motifs and/or domains or to arbitrary sizes, are within the scope of this application. Isolated peptidyl portions of SDF-1 can be obtained by screening peptides recombinantly produced from the corresponding fragment of the nucleic acid encoding such peptides. For example, an SDF-1 polypeptide may be arbitrarily divided into fragments of desired length with no overlap of the fragments, or preferably divided into overlapping fragments of a desired length. The fragments can be produced recombinantly and tested to identify those peptidyl fragments, which can function as agonists of native CXCR-4 polypeptides.

[0075] Variants of SDF-1 polypeptides can also include recombinant forms of the SDF-1 polypeptides. Recombinant polypeptides in some embodiments, in addition to SDF-1 polypeptides, are encoded by a nucleic acid that can have at least 70% sequence identity with the nucleic acid sequence of a gene encoding a mammalian SDF-1.

[0076] SDF-1 variants can include agonistic forms of the protein that constitutively express the functional activities of native SDF-1. Other SDF-1 variants can include those that are resistant to proteolytic cleavage, as for example, due to mutations, which alter protease target sequences. Whether a change in the amino acid sequence of a peptide results in a variant having one or more functional activities of a native SDF-1 can be readily determined by testing the variant for a native SDF-1 functional activity.

[0077] In some embodiments, SDF-1 includes a nucleic acid encoding any of the SDF-1 proteins described herein. The SDF-1 nucleic acid that encodes the SDF-1 protein can be a native or non-native nucleic acid and be in the form of RNA or in the form of DNA (*e.g.*, cDNA, genomic DNA, and synthetic DNA). The DNA can be double-stranded or single-stranded, and if single-stranded may be the coding (sense) strand or non-coding (anti-sense) strand. In one exemplary embodiment, SDF-1 that is loaded into and encapsulated by myocardial tissue targeting peptide conjugated nanoparticles can include an SDF-1 expressing plasmid DNA.

[0078] The nucleic acid coding sequence that encodes SDF-1 may be substantially similar to a nucleotide sequence of the SDF-1 gene, such as nucleotide sequence shown in SEQ ID NO: 6 and SEQ ID NO: 7. SEQ ID NO: 6 and SEQ ID NO: 7 comprise, respectively, the nucleic acid sequences for human SDF-1 and rat SDF-1 and are substantially similar to the nucleic sequences of GenBank Accession No. NM199168 and GenBank Accession No. AF189724. The nucleic acid coding sequence for SDF-1 can also be a different coding sequence which, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptide as SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5.

[0079] Other nucleic acid molecules that encode SDF-1 are variants of a native SDF-1, such as those that encode fragments, analogs and derivatives of native SDF-1. Such variants may be, for example, a naturally occurring allelic variant of a native SDF-1 gene, a homolog or ortholog of a native SDF-1 gene, or a non-naturally occurring variant of a native SDF-1 gene. These variants have a nucleotide sequence that differs from a native SDF-1 gene in one or more bases. For example, the nucleotide sequence of such variants can feature a deletion, addition, or substitution of one or more nucleotides of a native SDF-1 gene. Nucleic acid insertions are preferably of about 1 to 10 contiguous nucleotides, and deletions are preferably of about 1 to 10 contiguous nucleotides.

[0080] In other applications, variant SDF-1 displaying substantial changes in structure can be generated by making nucleotide substitutions that cause less than conservative changes in the encoded polypeptide. Examples of such nucleotide substitutions are those that cause changes in (a) the structure of the polypeptide backbone; (b) the charge or hydrophobicity of the polypeptide; or (c) the bulk of an amino acid side chain. Nucleotide substitutions generally expected to produce the greatest changes in protein properties are those that cause non-conservative changes in codons. Examples of codon changes that are likely to cause major changes in protein structure are those that cause substitution of (a) a hydrophilic residue (*e.g.*, serine or threonine), for (or by) a hydrophobic residue (*e.g.*, leucine, isoleucine, phenylalanine, valine or alanine); (b) a cysteine or proline for (or by) any other residue; (c) a residue having an electropositive side chain (*e.g.*, lysine, arginine, or histidine), for (or by) an electronegative residue (*e.g.*, glutamine or aspartine); or (d) a residue having a bulky side chain (*e.g.*, phenylalanine), for (or by) one not having a side chain, (*e.g.*, glycine).

[0081] Naturally occurring allelic variants of a native SDF-1 gene are nucleic acids isolated from mammalian tissue that have at least 70% sequence identity with a native SDF-1 gene, and encode polypeptides having structural similarity to a native SDF-1 polypeptide. Homologs of a native SDF-1 gene are nucleic acids isolated from other species that have at least 70% sequence identity with the native gene, and encode polypeptides having structural similarity to a native SDF-1 polypeptide. Public and/or proprietary nucleic acid databases can be searched to identify other nucleic acid molecules having a high percent (*e.g.*, 70% or more) sequence identity to a native SDF-1 gene.

[0082] Non-naturally occurring SDF-1 gene variants are nucleic acids that do not occur in nature (*e.g.*, are made by the hand of man), have at least 70% sequence identity with a native SDF-1 gene, and encode polypeptides having structural similarity to a native SDF-1 polypeptide. Examples of non-naturally occurring SDF-1 gene variants are those that encode a fragment of a native SDF-1 protein, those that hybridize to a native SDF-1 gene or a complement of to a native SDF-1 gene under stringent conditions, and those that share at least 65% sequence identity with a native SDF-1 gene or a complement of a native SDF-1 gene.

[0083] Nucleic acids encoding fragments of a native SDF-1 gene in some embodiments are those that encode amino acid residues of native SDF-1. Shorter oligonucleotides that encode or hybridize with nucleic acids that encode fragments of native SDF-1 can be used as

probes, primers, or antisense molecules. Longer polynucleotides that encode or hybridize with nucleic acids that encode fragments of a native SDF-1 can also be used in various aspects of the application. Nucleic acids encoding fragments of a native SDF-1 can be made by enzymatic digestion (*e.g.*, using a restriction enzyme) or chemical degradation of the full-length native SDF-1 gene or variants thereof.

[0084] Nucleic acids that hybridize under stringent conditions to one of the foregoing nucleic acids can also be used herein. For example, such nucleic acids can be those that hybridize to one of the foregoing nucleic acids under low stringency conditions, moderate stringency conditions, or high stringency conditions.

[0085] Nucleic acid molecules encoding a SDF-1 fusion protein may also be used in some embodiments. Such nucleic acids can be made by preparing a construct (*e.g.*, an expression vector) that expresses a SDF-1 fusion protein when introduced into a suitable target cell. For example, such a construct can be made by ligating a first polynucleotide encoding a SDF-1 protein fused in frame with a second polynucleotide encoding another protein such that expression of the construct in a suitable expression system yields a fusion protein.

[0086] The nucleic acids encoding SDF-1 can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The nucleic acids described herein may additionally include other appended groups such as imaging agents, or agents facilitating transport across a myocardial cell membrane or hybridization-triggered cleavage. To this end, the nucleic acids may be conjugated to another molecule, (*e.g.*, a peptide), hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

[0087] In some embodiments, an agent that causes, increases, and/or upregulates expression of SDF-1 can be loaded into and encapsulated by the nanoparticles of the invention. Such agents can be encapsulated either alone or in combination with an SDF-1 polypeptide or variant thereof described herein. For example, conjugated nanoparticles including SDF-1 can be systemically administered in combination with conjugated nanoparticles including an agent that causes, increases, and/or upregulates expression of SDF-1. Alternatively, conjugated nanoparticles including both SDF-1 and an agent that causes, increases, and/or upregulates expression of SDF-1 can be systemically administered to a subject for the treatment of a cardiomyopathy.

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[0088] The agent that causes, increases, and/or upregulates expression of SDF-1 can comprise natural or synthetic nucleic acids as described herein that are incorporated into recombinant nucleic acid constructs, typically DNA constructs, capable of introduction into and replication in the cells of the myocardial tissue. Such a construct can include a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given cell.

[0089] In one embodiment, a vector can comprise an SDF-1 plasmid. An SDF-1 plasmid can be loaded into and encapsulated by a conjugated nanoparticle and delivered to cells of the weakened region, ischemic region, and/or peri-infarct region of the myocardial tissue via systemic administration of the nanoparticle to a subject at an amount effective to improve at least one myocardial functional parameters, such as left ventricular volume, left ventricular area, left ventricular dimension, or cardiac function as well as improve the subject's 6-minute walk test (6MWT) or New York Heart Association (NYHA) functional classification. By delivering the nanoparticles into or about the periphery of the weakened region, ischemic region, and/or peri-infarct region of the myocardial tissue, it is possible to target the vector transfection rather effectively, minimize loss of the recombinant vectors, and enhance uptake of the SDF-1 plasmid by the cells. This method of administration enables local transfection of a desired number of cells, especially about the weakened region, ischemic region, and/or peri-infarct region of the myocardial tissue, thereby maximizing therapeutic efficacy of gene transfer, and minimizing the possibility of an inflammatory response to viral proteins.

[0090] In some embodiments, the SDF-1 delivered to myocardial tissue as described herein can be expressed at a therapeutically effective amount or dose in the weakened, ischemic, and/or peri-infarct region after transfection with the SDF-1 plasmid vector for greater than about three days. Expression of SDF-1 at a therapeutically effective dose or amount for greater three days can provide a therapeutic effect to weakened, ischemic, and/or peri-infarct region. Advantageously, the SDF-1 can be expressed in the weakened, ischemic, and/or peri-infarct region after transfection with the delivered SDF-1 plasmid vector at a therapeutically effective amount for less than about 90 days to mitigate potentially chronic and/or cytotoxic effects that may inhibit the therapeutic efficacy of the administration of the SDF-1 to the subject.

[0091] It will be appreciated that the amount, volume, concentration, and/or dosage of SDF-1 plasmid that is administered as part of a targeted nanoparticle composition to any one

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animal or human depends on many factors, including the subject's size, body surface area, age, the particular composition to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Specific variations of the above noted amounts, volumes, concentrations, and/or dosages of SDF-1 plasmid loaded into nanoparticles can be readily be determined by one skilled in the art using the experimental methods described below.

[0092] Optionally, other agents besides SDF-1 nucleic acids (*e.g.*, SDF-1 plasmids) can be loaded into a conjugated nanoparticle described herein and delivered to the weakened, ischemic, and/or peri-infarct region of the myocardial tissue to promote expression of SDF-1 from cells of the weakened, ischemic, and/or peri-infarct region. For example, agents that increase the transcription of a gene encoding SDF-1 increase the translation of an mRNA encoding SDF-1, and/ or those that decrease the degradation of an mRNA encoding SDF-1 could be used to increase SDF-1 protein levels. Increasing the rate of transcription from a gene within a cell can be accomplished by introducing an exogenous promoter upstream of the gene encoding SDF-1. Enhancer elements, which facilitate expression of a heterologous gene, may also be employed.

[0093] Other agents that can be loaded into and encapsulated by a conjugated nanoparticle of the present invention can include other proteins, chemokines, and cytokines, that when administered to the target cells can upregulate expression SDF-1 form the weakened, ischemic, and/or peri-infarct region of the myocardial tissue. Such agents can include, for example: insulin-like growth factor (IGF)-1, which was shown to upregulate expression of SDF-1 when administered to mesenchymal stem cells (MSCs) (Circ. Res. 2008, Nov 21; 103(11):1300-98); sonic hedgehog (Shh), which was shown to upregulate expression of SDF-1 when administered to adult fibroblasts (Nature Medicine, Volume 11, Number 11, Nov. 23); transforming growth factor β (TGF- β); which was shown to upregulate expression of SDF-1 when administered to human peritoneal mesothelial cells (HPMCs); IL-1 β , PDGF, VEGF, TNF- α , and PTH, which are shown to upregulate expression of SDF-1, when administered to primary human osteoblasts (HOBs) mixed marrow stromal cells (BMSCs), and human osteoblast-like cell lines (Bone, 2006, Apr; 38(4): 497-508); thymosin β 4, which was shown to upregulate expression when administered to bone marrow cells (BMCs) (Curr. Pharm. Des. 2007; 13(31):3245-51; and hypoxia inducible factor 1 α (HIF-1), which was shown to upregulate expression of SDF-1 when administered to bone marrow derived

progenitor cells (Cardiovasc. Res. 2008, E. Pub.). These agents can be used to treat specific cardiomyopathies where such cells capable of upregulating expression of SDF-1 with respect to the specific cytokine are present or administered.

[0094] Pharmaceutical compositions in accordance with the methods of the present invention will generally include an amount of loaded conjugated nanoparticles described herein admixed with an acceptable pharmaceutical diluent or excipient, such as a sterile aqueous solution, to give a range of final concentrations, depending on the intended use. The techniques of preparation are generally well known in the art as exemplified by Remington's Pharmaceutical Sciences, 16th Ed. Mack Publishing Company, 1980, incorporated herein by reference. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards.

[0095] The pharmaceutical nanoparticle compositions can be in a unit dosage injectable form (*e.g.*, solution, suspension, and/or emulsion). Examples of pharmaceutical formulations that can be used for injection include sterile aqueous solutions or dispersions and sterile powders for reconstitution into sterile injectable solutions or dispersions. The carrier can be a solvent or dispersing medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, liquid polyethylene glycol, and the like), dextrose, saline, or phosphate-buffered saline (*e.g.*, PBS), suitable mixtures thereof and vegetable oils.

[0096] In an exemplary embodiment, parenteral formulations of pharmaceutical conjugated nanoparticle include freeze dried (lyophilized) nanoparticles stored in a desiccator at 4° C and reconstituted in a suitable medium (*e.g.*, PBS) prior to systemic administration to a subject.

[0097] Proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Nonaqueous vehicles such as cottonseed oil, sesame oil, olive oil, soybean oil, corn oil, sunflower oil, or peanut oil and esters, such as isopropyl myristate, may also be used as solvent systems for compound compositions.

[0098] Additionally, various additives, which enhance the stability, sterility, and isotonicity of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol,

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sorbic acid, and the like. In many cases, it will be desirable to include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to methods described herein, however, any vehicle, diluent, or additive used would have to be compatible with the conjugated nanoparticle compositions described herein.

[0099] The following examples are for the purpose of illustration only and are not intended to limit the scope of the claims, which are appended hereto.

EXAMPLE 1

IDENTIFICATION OF TARGETING PEPTIDES

[00100] In this example, the main focus was to identify one or a few ligands that have a high affinity for infarct tissue and are specific to the heart. This can then be used to tag polymeric devices, or the naked plasmids to infuse therapeutic genes to the ischemic myocardium.

[00101] We infused the circular C7C peptide library in M13bacteriophages into rodents that exhibited chronic heart failure. 1.5 hours post infusion we removed the heart, separated the infarcted and border region, and eluted the phages in this region of the heart. This was amplified and panned for a second round. After 3 rounds of panning and sequencing of the peptides with affinity to the infarcted region of the heart, we identified the sequence to be a 7 peptide sequence of RQPRMKR (RR peptide, SEQ ID NO:1) flanked on either sides by cysteines to provide a circular CRQPRMKRC (SEQ ID NO:2) ligand with selective affinity to the heart. On infusing this peptide tagged with a fluorescent label, we confirmed the targeting nature of this peptide with immunohistochemistry studies.

Mechanism of phage panning

[00102] The Ph.D. C7C library consists of random 7-mer peptides flanked by cysteines in order to conserve the 7-mer peptide by giving it a circular conformation. The cysteine residues are oxidized during phage assembly, and thus allow for a di-sulfide link that brings the 7-mer peptide to a circular construct, conserving its sequence. These 7-mer peptides are fused to the minor coat protein of the M13 bacteriophage via a linker sequence Gly-Gly-Gly-Ser. These variant libraries are expressed on the outside of the phage, while the genetic

material encoding the 7 mer is inside the phage particle. The presence of the peptide on the surface, bound to the phage, creates a link that can bind the phage to selective targets, antibodies, receptors enzymes etc.

[00103] *In vitro* phage panning is carried out when multiple copies of each variant peptide is exposed to a surface antigen, the unbound phage is washed away and the resulting phage is eluted, amplified and exposed to a second surface with the antigen. Each such round is called panning. After 3 to 4 rounds of panning, an exclusive sequence is identified by DNA sequencing and ELISA, which binds to the antigen.

[00104] *In vivo* phage panning refers to an extension of the *in vitro* phage panning, to identify potential ligands that bind to organs. Phage with its variant libraries is circulated through an animal, following infusion via a vein. The organs are harvested and the bound phage is eluted, amplified and identified.

Methods

Phage Panning

[00105] M13 phage library Ph.D.-7 (M13-based, complexity 2.8×10^9 transformants; New England BioLabs, Schwalbach, Germany) which displays random 7-mer peptides at the N-terminus of the pIII protein (high stringency: only 3–5 copies per phage particle) was used. 1.2×10^{11} pfu of phage library in 200 μ l saline was delivered to three anesthetized Lewis rats (8–12 weeks old), one month post infarction via LAD ligation. LAD ligation is described above. These animals were sacrificed, 1.5 hours post infusion. Following careful dissection to avoid any possible puncture of gastro-intestinal tract and exsanguinations by cutting jugular veins, the rats were perfused through the heart with 120ml of saline to remove all blood. The hearts were removed and washed in ice cold saline. Tissues were weighed and homogenized in 2 ml of tissue suspension buffer (DMEM, with 1% BSA and 10% protease inhibitors). Bound phage was eluted from this with glycine and stabilized with Tris. This was amplified with E Coli. 1.2×10^{11} pfu of amplified phage was re-infused via tail veins. This process was repeated three times. At the end of the 3rd round, eluted phage was sequenced and identified.

Peptide Synthesis and Analysis

[00106] The RQPRMKR (SEQ ID NO:1) peptide was synthesized by the Protein Core lab at the Cleveland Clinic and confirmed by mass spectrometry. This peptide was tagged with a His tag. A 6-His peptide was attached to the N terminal end, thus synthesizing a 6-His-RQPRMKR peptide (RR peptide) (SEQ ID NO:1).

[00107] Rats were infarcted via LAD ligation, as described before. One month post ligation, the RR peptide was infused via the tail vein. 1.5 hours later, the animal was sacrificed and the heart, lung, liver, spleen and kidney was excised, and washed in ice cold PBS.

Immunohistochemistry

[00108] Organs were embedded in OCT compound and were frozen immediately. The tissue samples were stored for long periods of time at -80°C. Frozen samples were cut in a cryotome at 5 micron thickness and mounted on slides. Tissue specimens were fixed with paraformaldehyde and incubated with 1% normal blocking serum with goat and donkey serum in PBS for 60 min to suppress nonspecific binding of IgG. Slides were then incubated for 60 min with mouse monoclonal anti His antibody (Abcam Cambridge, MA) at 1:200 dilutions in blocking buffer with serum. Slides were then washed with phosphate-buffered saline (PBS) and incubated for 45 min, with IgG goat anti-mouse Alexa fluor 488 (Molecular Probes, Invitrogen, Carlsbad, CA) at 1:800 dilution in blocking buffer with serum in a dark chamber. After washing extensively with PBS, coverslips were mounted with aqueous mounting medium. (Vectashield Mounting Medium with DAPI H-1200; Vector Laboratories, Burlingame, CA, USA).

Results

Identification of targeting peptide

[00109] In vivo phage panning was performed in rats with LAD ligation, one month post infarction. Infarction in the hearts was confirmed by echocardiography. 1.2×10^{12} particles were infused via the tail vein. 1.5 hours later, when the particles have had enough time to circulate through the body, hearts were excised. The blanched region was identified as the infarct zone. The region in between this zone and the healthy tissue is termed the border zone. Tissue sections were washed in ice cold saline, and the blanched region was sectioned

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out. Tissues were homogenized and the phage was eluted. Phage was sequenced using primers provided in the kit. Three colonies were picked after each round of panning. After three rounds of panning, a common sequence of Arg-Glu-Pro-Arg-Met-Lys-Arg (RQPRMKR) (SEQ ID NO:1) was identified.

Synthetic Peptide Formulation and Analysis

[00110] This peptide was synthesized at the CC Proteomics Core lab. The peptides were synthesized with a fluorescent tag attached to one end. A (His)₆ – RQPRMKR (SEQ ID NO:1) peptide was synthesized and the purity was checked by reverse phase liquid chromatography. The mass was determined using a mass spectrometer and was recorded at 1794.5923 units using a 4700 Reflector Spectrometer.

In Vivo Expression Profile of the RR peptide

[00111] The RR peptide tagged with His was infused at 200 µg of peptide in 200 µl of PBS into tail veins in rodents, one month after LAD ligation. Prior to infusion, rats hearts were imaged via echocardiography to ensure an akinetic wall motion and a loss in fractional shortening (<30%). The rats were then perfused with saline, and the organs were excised and embedded in OCT compound. Sections were made and the tissue samples were stained with fluorescent antibody markers against His and Dapi for nuclear detection. Heart sections were stained positive against the His antibody, thereby confirming the presence of the (His)₆ – RR peptide. All other organs, lungs, spleen, liver and kidney were stained negative for His, thus confirming the absence of this peptide in all other organs (Fig. 1).

[00112] The RR peptide specifically targets the heart tissues and has high affinity to the infarct region. This peptide may be used as a targeting molecule to direct therapeutic drugs and genes to the infarct region.

BLAST Analysis of the targeting peptide

[00113] The RQPRMKR (SEQ ID NO: 1) sequence with the genomic code of agg cag ccg cgc atg aag cgg (SEQ ID NO: 3) was compared against all the known sequences in the rat genome using the BLAST software. There were some similarities in the sequence code with other known protein sequences such as FAS Associated Factor -1 (FAF-1) and another protein Sodium-dependent phosphate transporter 1 or Solute carrier family 20 member 1

a.k.a. Phosphate transporter 1 (PiT-1). But neither of these sequences possesses all the 7-mer sequence, therefore a conclusive evidence of either of these two proteins is lacking.

ENGINEERED NANOPARTICLES AS CARRIERS FOR SYSTEMIC DELIVERY

[00114] Our focus was to engineer a polymeric carrier that is capable of targeting the ischemic myocardium and delivering therapeutic drugs to it. PLGA polymeric devices may be able to encapsulate therapeutic genes and deliver them safely to tissues via the systemic circulation.

[00115] We hypothesized that encapsulating the gene of interest into PLGA polymeric particles, tagged with the RQPRMKR ("RR", SEQ ID NO:1) peptide will be able to provide a targeting encapsulated drug capable of homing to the ischemic myocardium via systemic delivery. The targeting peptide, RR, when tagged to the polymeric complex, will be viable and in its active form, and will be able to direct the polymeric complex to the injured myocardium. The PLGA polymer will then be able to release the encapsulated material into the area of injury.

[00116] 6 Coumarin (6C) is a lipophilic fluorescent dye that can be viewed by confocal microscopy, and its fluorescence activity can be accurately measured by High Performance Liquid Chromatography (HPLC) analysis. Our focus was to design a nanoparticulate carrier capable of encapsulating 6C, a fluorescent dye, and delivering it to the myocardium, with the help of a tagging peptide. The uptake and *in vivo* trafficking would then be monitored to determine the dose, time, and other aspects associated with this delivery system.

[00117] In order to determine the release and uptake of nanoparticles *in vitro*, we added nanoparticles with or without the homing peptide to cardiac fibroblasts seeded on 6 well plates. As expected, we did not see a difference in fluorescence between the two groups. The uptake took place in about 30 minutes and stayed for more than 24 hours. *In vivo*, we observed a greater uptake of nanoparticles, as determined by the amount of fluorescence in various organs, in the injured myocardium, as opposed to the healthy myocardium. RR peptide helps target the encapsulated material to the injured myocardium.

[00118] On replacing the 6C dye with a therapeutic gene, such as SDF-1 we may be able to show improved cardiac benefit via systemic delivery, capable of translating this complex, to patients with chronic heart failure.

Methods

Materials

[00119] BSA (Fraction V) and PVA (average molecular weight, 30,000–70,000) were purchased from Sigma (St. Louis, MO). 6-Coumarin was purchased from Polysciences (Warrington, PA). PLGA (50:50 lactide–glycolide ratio, 143,000 Da, viscosity 0.87dl/g) was purchased from Birmingham Polymers (Birmingham, AL). RR-peptide of the sequence (His)₆- Arg-Glu-Pro-Arg-Met-Lys-Arg (molecular weight 1784) (SEQ ID NO.1) was custom synthesized by Cleveland Clinic Proteomics Core (Cleveland, OH). Denacol_ EX-521 (Pentaepoxy, molecular weight 742) was a gift from Nagase Chemicals Ltd (Tokyo, Japan). Zinc tetrahydrofluoroborate hydrate, poly(vinyl alcohol) (PVA, average molecular weight 30,000–70,000), dextran, boric acid, and ethanol were obtained from Sigma Chemical Co. (St. Louis, MO). Chloroform was obtained from Fisher Scientific (Pittsburgh, PA).

Formulation of 6C loaded PLGA nanoparticles

[00120] Nanoparticles containing BSA and 6-coumarin were formulated using a double emulsion-solvent evaporation technique as described previously. An aqueous solution of BSA (60 mg/ml, 1 ml) was emulsified in a PLGA solution (180 mg in 6ml chloroform) containing 6-coumarin (100µg) using a probe sonicator (55W for 2 min) (Sonicator® XL, Misonix, NY). The water-in-oil emulsion formed was further emulsified into 50 ml of 2.5% w/v aqueous solution of PVA by sonication (55W for 5 min) to form a multiple water-in-oil-in-water emulsion. The multiple emulsion was stirred for ~18 h at room temperature followed by for 1 h in a desiccator under vacuum to remove the residual chloroform. Nanoparticles were recovered by ultracentrifugation (35,000 rpm for 20 min at 4°C, Optima™ LE-80K, Beckman, Palo Alta, CA), washed two times with distilled water to remove PVA, untrapped BSA and 6-coumarin, and then lyophilized (–80 °C and <10_m mercury pressure, Sentry™, Virtis, Gardiner, NY) for 48 h to obtain a dry powder. Dry lyophilized nanoparticle samples were stored in a desiccator at 4°C and were reconstituted in a suitable medium (buffer or cell culture medium) prior to an experiment.

Conjugation of targeting peptide

[00121] The peptide to be conjugated is the RQPRMKR (SEQ ID NO:1) peptide, identified via phage panning experiments. Prior to conjugation, the peptide is tagged with

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(His)₆ residues in its N terminal. The peptide was conjugated to the nanoparticles in two steps.

[00122] Step 1: Surface activation step: nanoparticles were suspended in borate buffer (50mM, pH 5.0) by sonication for 30 sec on an ice bath. This is followed by the addition of DENACOL (40 mg), an epoxy that helps conjugation of the peptide on the surface and the catalyst zinc tetrahydrofluroborate hydrate (50 mg) also dissolved in an equal volume of buffer to the NP solution. This mixture was stirred gently for 30 minutes at 37°C. NPs were separated by ultracentrifugation at 30000 rpm for 20 minutes at 4°C. Any unreacted DENACOL is removed by multiple wash steps.

[00123] Step 2 : Conjugation of peptide: Surface activated NPs were suspended in borate buffer (4 ml) and stirred into a solution containing three different initial amounts of peptides; 250ug, 500ug and 1 mg in borate buffer. This reaction is carried out for 2 hours at 37°C. The unreacted peptide was removed by ultracentrifugation and the final nanoparticles suspension was lyophilized for 48 hours.

Nanoparticle characterization

Particle size and surface charge (zeta potential)

[00124] Particle size and size distribution were determined by photon correlation spectroscopy (PCS) using quasi-elastic light scattering equipment (ZetaPlus™ equipped with particle sizing mode, Brookhaven Instrument Corp., Holtsville, NY). A dilute suspension (100µg/ml) of nanoparticles was prepared in double distilled water and sonicated on an ice bath for 30 s. The sample was subjected to particle size analysis. Zeta potential of nanoparticles in 0.001M double distilled water, was determined using ZetaPlus™ in the zeta potential analysis mode.

In vitro expression and uptake of 6-coumarin

[00125] Rat cardiac fibroblasts (abbreviated as rCFs,) cultured in DMEM Medium with 10% fetal bovine serum (Gibco, NY) and 1% penicillin G and streptomycin (Gibco BRL, Grand Island, NY), were used for all the cell culture studies. The cells were seeded at 200,000 cells per well/1 ml (50% confluency) in a 6-well plate and allowed to attach overnight. Nanoparticles were added at different doses in 1 ml of the medium and incubated for 2 days. Untreated cells (plain medium) were used as a control. At the end of the

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incubation period, cells were washed twice with phosphate buffered saline (PBS, pH 7.4) to remove nanoparticles and 1 ml of fresh medium was added to each well. The uptake of the nanoparticles in the cells was determined using optical microscopy studies, and high performance liquid chromatography (HPLC) analysis

Extraction and quantitation of 6C fluorescence in cells

[00126] Cells were washed with PBS three times and were solubilized in 1% Triton X 100 solution. This suspension was stored at -80°C overnight and lyophilized for 48 hours. Lyophilized cell lysates were reconstituted in methanol (500ul) and incubated at 37°C for 18 hours to extract the dye. Samples were centrifuged at 14000 rpm for 10 minutes at 4°C to remove cell debris. Supernatants were collected for HPLC analysis. A standard curve is obtained by adding 8 µg to 40 µg of nanoparticles in Triton X 100 solution and lyophilized as the cell samples.

Confocal Microscopy Analysis

[00127] rCFs were plated on coverslips in 6 well plates at 50,000 cells per well (50% confluency) in 1 ml of culture medium and allowed to attach for 24 h. Cells were then incubated with 6-coumarin loaded nanoparticle suspension (200µg/ml) in growth medium for 60 min. Cells were then washed twice with PBS and visualized with a Zeiss Confocal LSM410 microscope equipped with 488 nm excitation laser (Carl Zeiss Microimaging, Inc., Thornwood, NY).

In vivo Expression Analysis

[00128] Rats were infarcted via LAD ligation, as described above. 4 weeks post infarction, nanoparticles with or without the tagging peptide was infused via the tail vein at 200 µg of particles per animal in 200µl of PBS. The nanoparticles were allowed to circulate overnight, after which the animals were sacrificed by de-sanguination by perfusion with saline, and the organs (heart and liver) were excised. Heart tissues were divided based on the region of infarct or the healthy region. The wet weight of the tissues was recorded. Samples were homogenized in 2 ml of distilled water using a tissue homogenizer. The homogenized samples were then lyophilized for 48 hours. The 6C dye from the homogenized tissue was extracted by shaking with 5 ml of methanol at 37°C for 24 hours at 100 rpm using an Environ orbital shaker. Standard curve was obtained by samples varying from 8 µg to 40µg of the

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nanoparticles in methanol. Tissue extracts were centrifuged at 14000 rpm for 10 minutes to remove cell debris. The supernatant was collected and this was used for HPLC analysis.

HPLC Analysis

[00129] A Shimadzu HPLC system (Shimadzu Scientific Instruments, Columbia, MO) fitted with an SCL-10A system controller, an SIL-10A auto-injector, LC-10AT pump, a RF-10A XL fluorescence detector, and Class VP chromatography data system software version 4.2 was used. A Nova pak C-8 column with spherical particles (4 mm) and pore size of 60Å (Waters, Milford, MA) and the mobile phase consisting of acetonitrile /water (65:35) containing 5mM sodium salt of 1-heptane sulfonic acid (Aldrich, Milwaukee, WI) set at a flow rate of 0.8 ml/min were used to resolve the dye peak. Excitation and emission wavelengths were 450 and 490 nm respectively. A 20 ml aliquot of each sample was injected into HPLC. The retention time of 6-coumarin was 2.6 min. The amount of nanoparticles in different tissue samples was determined from a calibration curve plotted between the fluorescent intensity and the amount of fluorescently labeled nanoparticles that were treated similar to the tissue samples. There was no background peak for the control tissue (without nanoparticles). The assay is sensitive enough to detect as low as 5 ng nanoparticles in the tissue samples.

Results

Formulation of Nanoparticles

[00130] Nanoparticles with 6 Coumarin as a fluorescent marker and BSA as a model protein were formulated. Nanoparticles had a typical protein loading of 20% (w/w). As in, 20 mg of BSA was present in 100 mg of nanoparticles.

Particle Size Analysis and Zeta Potential

[00131] Particle size distribution was analyzed by dynamic light scattering technique. The size of the unconjugated PLGA polymer encapsulating the 6C dye in BSA was found to be 0.095 microns or 95 ± 12.3 nm. The conjugated PLGA polymer with the tagging peptide on the surface had an average size of 103 ± 11 nm indicating uniform particle size distribution. When these particle sizes were analyzed before sonicating them, the mean particle size was 600 ± 350 nm indicating the importance of sonicating on these particles.

[00132] The zeta potential, corresponding to the surface charge was recorded at -14.72 ± 0.95 mV for the unconjugated particles and -11.04 ± 6.22 mV for the conjugated particles.

[00133] Nanoparticles were resuspended easily after lyophilization to form a stable colloidal dispersion without any change in size or content. In previous studies, it has been shown that 99.4% of the entrapped dye remains associated with the nanoparticles even after 48 h of *in vitro* release study under constant conditions. Therefore, these nanoparticles serve as a good model formulation to study the tissue uptake *in vivo* in acute experiments. Furthermore, one can use these nanoparticles to quantitate the uptake as well as to study their localization in the cells/tissue by fluorescence microscopy

In vitro Expression Analysis

Dose Analysis

[00134] Rat cardiac fibroblasts were cultured in DMEM Medium with 10% fetal bovine serum and 1% penicillin and streptomycin. The cells were seeded at 50,000 cells per well in 1 ml of culture medium (50% confluency) in a 6-well plate and allowed to attach overnight. Nanoparticles with the conjugated peptides were added at different doses ranging between 10 μ g of nanoparticles to 1 mg of nanoparticles per well in 1 ml of the medium and incubated overnight. At the end of the incubation period, cells were washed twice with phosphate buffered saline (PBS, pH 7.4) to remove nanoparticles and 1 ml of fresh medium was added to each well. The cells were then viewed using optical microscopy (Fig. 2).

[00135] When less than 100 μ g of particles were added to the wells, the signal obtained was almost inconceivable. On the other hand a strong signal was observed at 500 μ g of particles and 1 mg of particles per well. A minimum of 200 μ g per well of particles are required for an observable signal. For future studies 200 μ g of particles per well was used.

Time for Uptake

[00136] Rat cardiac fibroblasts were cultured in DME Medium with 10% fetal bovine serum and 1% penicillin and streptomycin. The cells were seeded at 50,000 cells per well/1 ml of culture medium (50% confluency) in a 6-well plate and allowed to attach overnight. Nanoparticles with and without the conjugated peptides were added at 200 μ g of nanoparticles per well in 1 ml of the medium and incubated for varying lengths of time varying between 30 minutes and 24 hours to determine the optimum time for uptake.

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Unconjugated nanoparticles were used as controls. At the end of the incubation period, cells were washed twice with phosphate buffered saline (PBS, pH 7.4) to remove nanoparticles and 1 ml of fresh medium was added to each well. The cells were then viewed using optical microscopy.

[00137] When the cells were analyzed at 30 minutes or less, no significant signal was observed. However, after 1 hour there was an observable signal that increased for upto 3 hours, following which the signal remained constant for up to 24 hours. For future *in vitro* studies, nanoparticles will be added for a period of 2 hours before it is washed away. (Fig. 3)

[00138] As expected, there was no significant difference in signal between the two nanoparticles types, whether conjugated or un-conjugated. Particles were added to the wells and did not have to target any specific cell type. In fact, there seemed to be a slight increase in particle uptake with un-conjugated particles. (Fig. 4) Therefore in order to address this, a quantitative study was performed with conjugated and un-conjugated particles, at 200 μg of particles per well following 2 hours of uptake.

HPLC Analysis with *in vitro* particle study

[00139] Rat cardiac fibroblasts were cultured in the appropriate medium at 50,000 cells per well/1 ml of culture medium (50% confluency) in a 6-well plate and allowed to attach overnight. Nanoparticles with and without the conjugated peptides were added at 200 μg of nanoparticles per well in 1 ml of the medium and incubated 2 hours. Unconjugated nanoparticles were used as controls. At the end of the incubation period, cells were washed twice with phosphate buffered saline (PBS, pH 7.4) to remove nanoparticles and 1 ml of fresh medium was added to each well and were solubilized in 1% Triton X 100 solution. This solution was lyophilized for 48 hours, and cell lysates were reconstituted in methanol (500 μl) and incubated at 37°C for 18 hours to extract the dye. Samples were centrifuged at 14000 rpm for 10 minutes at 4°C to remove cell debris. Supernatants were collected for HPLC analysis. A Standard curve was obtained by adding 8 μg to 40 μg of nanoparticles in Triton X 100 solution and lyophilized as with the cell samples.

[00140] Following the HPLC analysis, the amount of fluorescent dye extracted from the un-conjugated particles was 14.89 ± 2.1 μg per well and the amount of 6C dye extracted from conjugated nanoparticles was 11.97 ± 3.24 μg per well. There is a slight decrease in the loading of conjugated particles that may be attributed to the slightly smaller sizes as

compared to the un-conjugated particles. However, this difference is not significant. However, when unsonicated 6C unconjugated nanoparticles were used, this uptake dropped to almost half, indicating the importance of particle size on uptake. (Fig. 5)

In vivo nanoparticle uptake in rodents

[00141] 4 weeks post infarction via LAD ligation in rodents, nanoparticles with or without the tagging peptide was infused via the tail vein at 200 µg of particles per animal in 200µl of PBS. The nanoparticles were allowed to circulate overnight, and the organs (heart and liver) were excised. Heart tissues were divided between infarcted and healthy regions. The wet weight of the tissues was recorded. Samples were homogenized, lyophilized and the 6C dye from the homogenized tissue was extracted by shaking with 5 ml of methanol at 37°C for 24 hours at 100 rpm. Tissue extracts were centrifuged at 14000 rpm for 10 minutes to remove cell debris. The supernatant was collected and this was used for HPLC analysis. Standard curve was obtained by samples varying from 8 µg to 40µg of the nanoparticles in methanol.

[00142] On analyzing the HPLC results, it was observed that infarcted tissue regions had more 6 C extracted from them, as compared to healthy tissues (n=3) (Fig. 6). Also, the amount of fluorescence intensity observed in the infarcted region of the heart increased when nanoparticles with the homing peptide were infused as compared to when nanoparticles alone were used. This increase in fluorescence intensity was significant, with a p value of < 0.01 (Fig. 7). When particles were conjugated with the ischemic heart targeting peptide, they travelled to the infarcted region of the heart.

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Having described the invention, we claim:

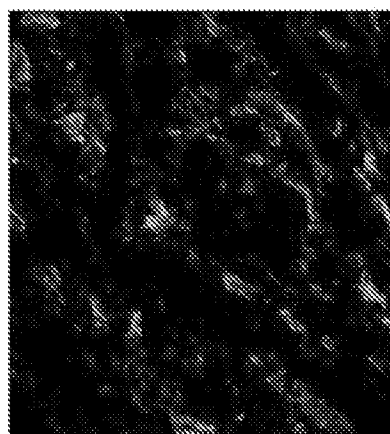
1. A myocardial tissue targeting peptide consisting of about 5 to about 25 amino acids, wherein at least a portion of the amino acid sequence of the peptide is homologous to at least 5 consecutive amino acids of SEQ ID NO:1.
2. The myocardial tissue targeting peptide of claim 1, wherein the peptide includes an amino acid sequence consisting of SEQ ID NO: 1.
3. The myocardial tissue targeting peptide of claim 1, wherein the peptide includes an amino acid sequence consisting of SEQ ID NO: 2.
4. The myocardial tissue targeting peptide of claim 1, wherein the peptide is selective for a weakened, ischemic, and/or peri-infarct region of mammalian myocardial tissue.
5. A method of delivering a therapeutic agent to myocardial tissue of a subject, the method comprising administering to the subject a nanoparticle, the nanoparticle comprising a polymeric carrier and a therapeutic agent, wherein the nanoparticle is conjugated to a myocardial tissue targeting peptide, the myocardial tissue targeting peptide consisting of about 5 to about 25 amino acids, wherein at least a portion of the amino acid sequence of the peptide is homologous to at least 5 consecutive amino acids of SEQ ID NO:1.
6. The method of claim 5, wherein the myocardial tissue targeting peptide includes an amino acid sequence consisting of SEQ ID NO: 1.
7. The method of claim 5, wherein the myocardial tissue targeting peptide includes an amino acid sequence consisting of SEQ ID NO: 2.
8. The method of claim 5, wherein the nanoparticle is administered systemically.

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9. The method of claim 8, wherein the myocardial tissue targeting peptide is selective for a weakened, ischemic, and/or peri-infarct region of mammalian myocardial tissue.
10. The method of claim 5, the polymeric carrier comprising PLGA.
11. The method of claim 5, wherein the therapeutic agent is encapsulated by the nanoparticle.
12. The method of claim 5, the therapeutic agent comprising a cytokine.
13. The method of claim 12, the cytokine comprising SDF-1.
14. The method of claim 5, the therapeutic agent comprising a nucleic acid.
15. The method of claim 14 the therapeutic agent comprising an SDF-1 expressing plasmid DNA.
16. A method of treating a cardiomyopathy in a subject comprising:
administering systemically to a subject a therapeutically effective amount of nanoparticles, the nanoparticles comprising a polymeric carrier and SDF-1, wherein the nanoparticle is conjugated to a myocardial tissue targeting peptide, the myocardial tissue targeting peptide consisting of about 5 to about 25 amino acids, wherein at least a portion of the amino acid sequence of the peptide is homologous to at least 5 consecutive amino acids of SEQ ID NO:1, wherein the therapeutically effective amount of nanoparticles effective to cause functional improvement in at least one of the following parameters: left ventricular volume, left ventricular area, left ventricular dimension, cardiac function, 6-minute walk test, or New York Heart Association (NYHA) functional classification.
17. The method of claim 16, wherein the myocardial tissue targeting peptide includes an amino acid sequence consisting of SEQ ID NO: 1.

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18. The method of claim 16, wherein the myocardial tissue targeting peptide includes an amino acid sequence consisting of SEQ ID NO: 2
19. The method of claim 16, wherein the myocardial tissue targeting peptide is selective for a weakened, ischemic, and/or peri-infarct region of mammalian myocardial tissue.
20. The method of claim 16, the polymeric carrier comprising PLGA.
21. The method of claim 16, wherein the SDF-1 is encapsulated by the nanoparticle.
22. The method of claim 16, the SDF-1 comprising an SDF-1 expressing plasmid DNA.



Heart



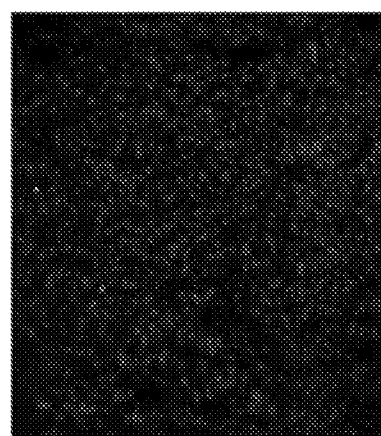
Lung



Kidney

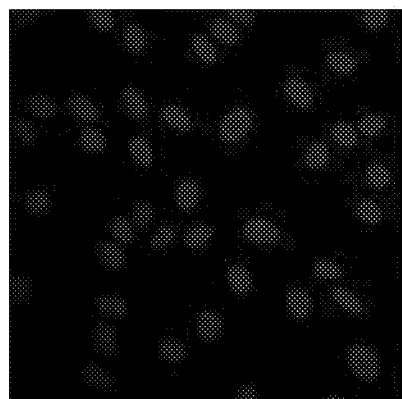


Spleen

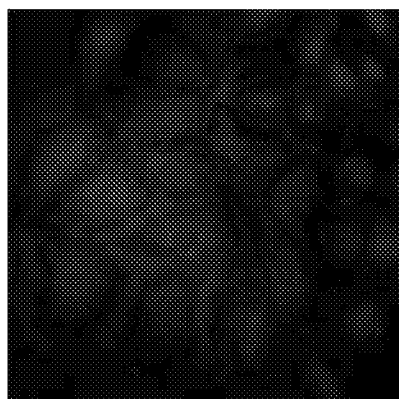


Liver

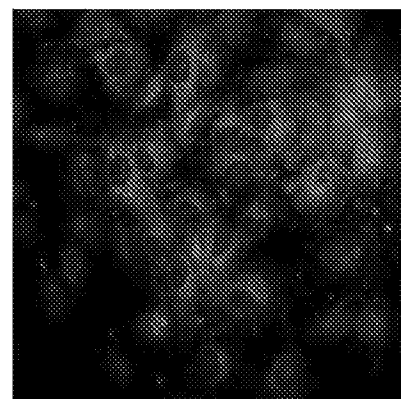
Fig. 1



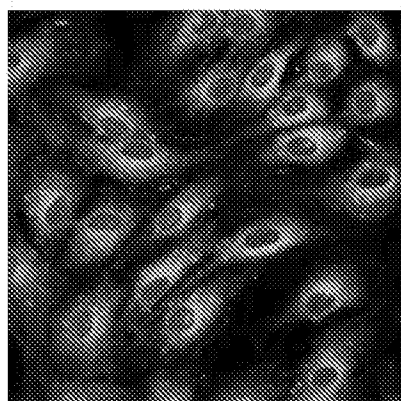
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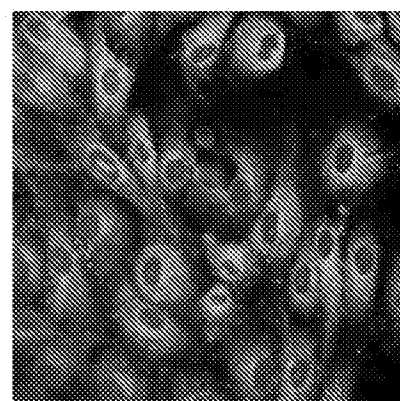
50 µg



100 µg



500 µg



1000 µg

Fig. 2

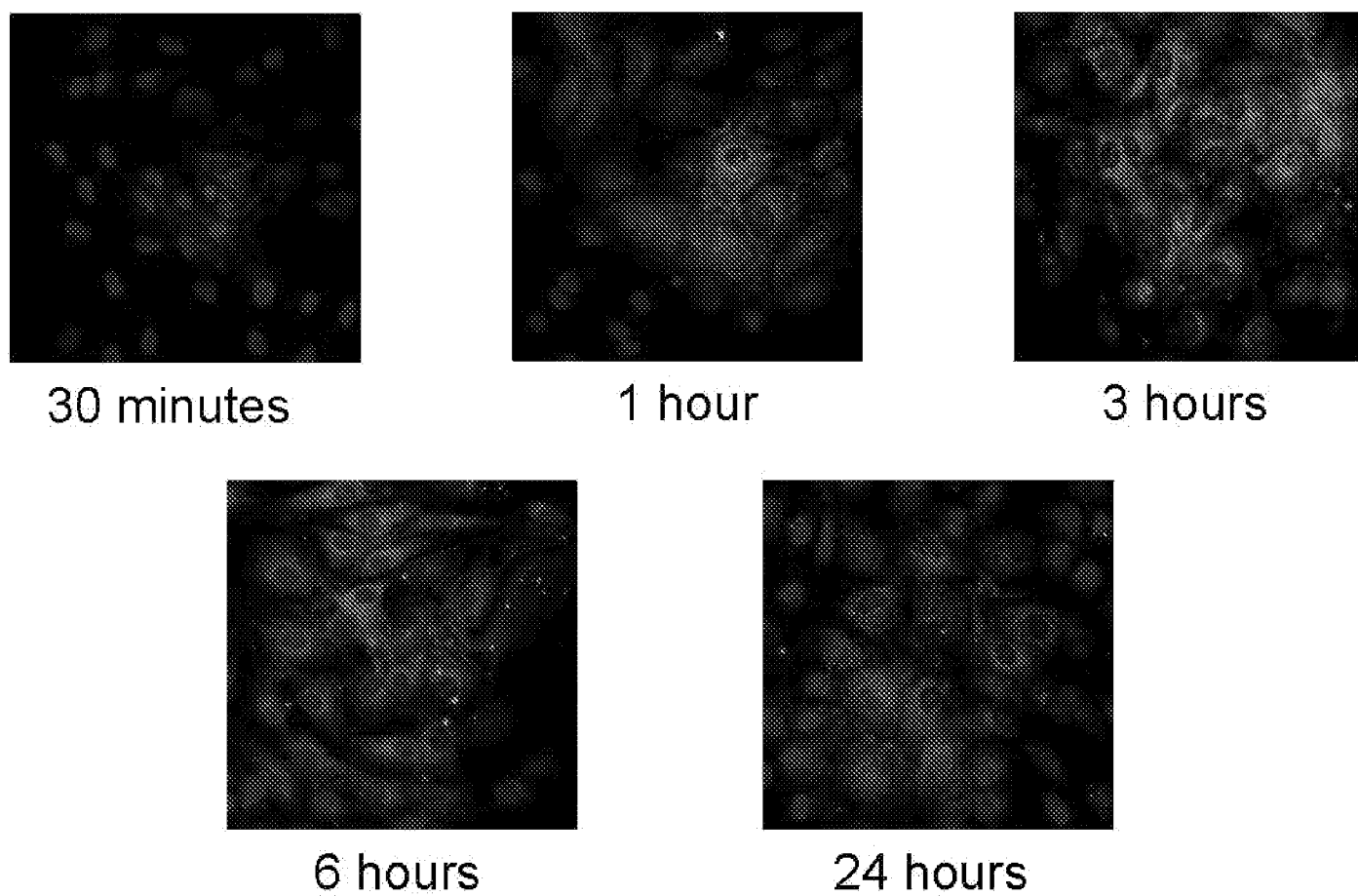
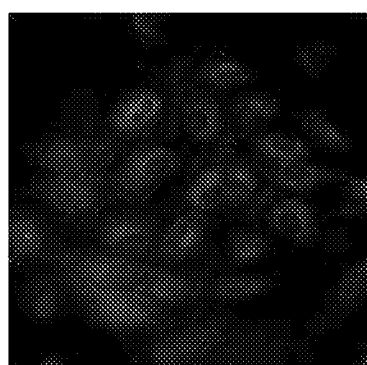
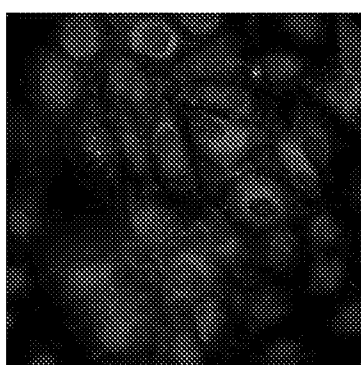


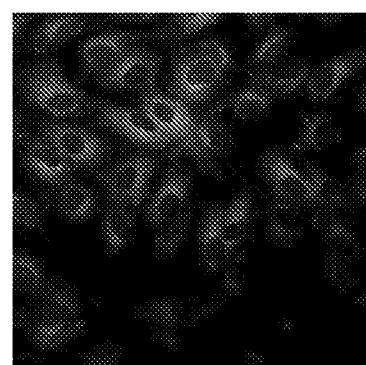
Fig. 3



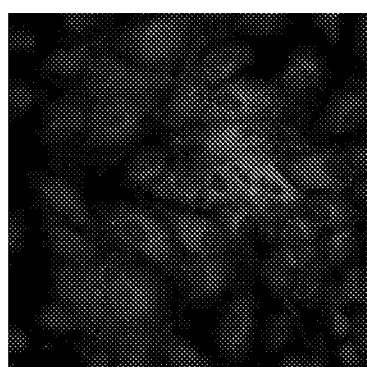
30 minutes



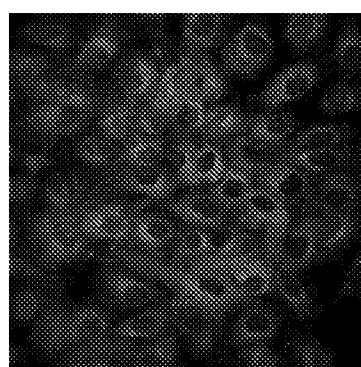
1 hour



3 hours



6 hours



24 hours

Fig. 4

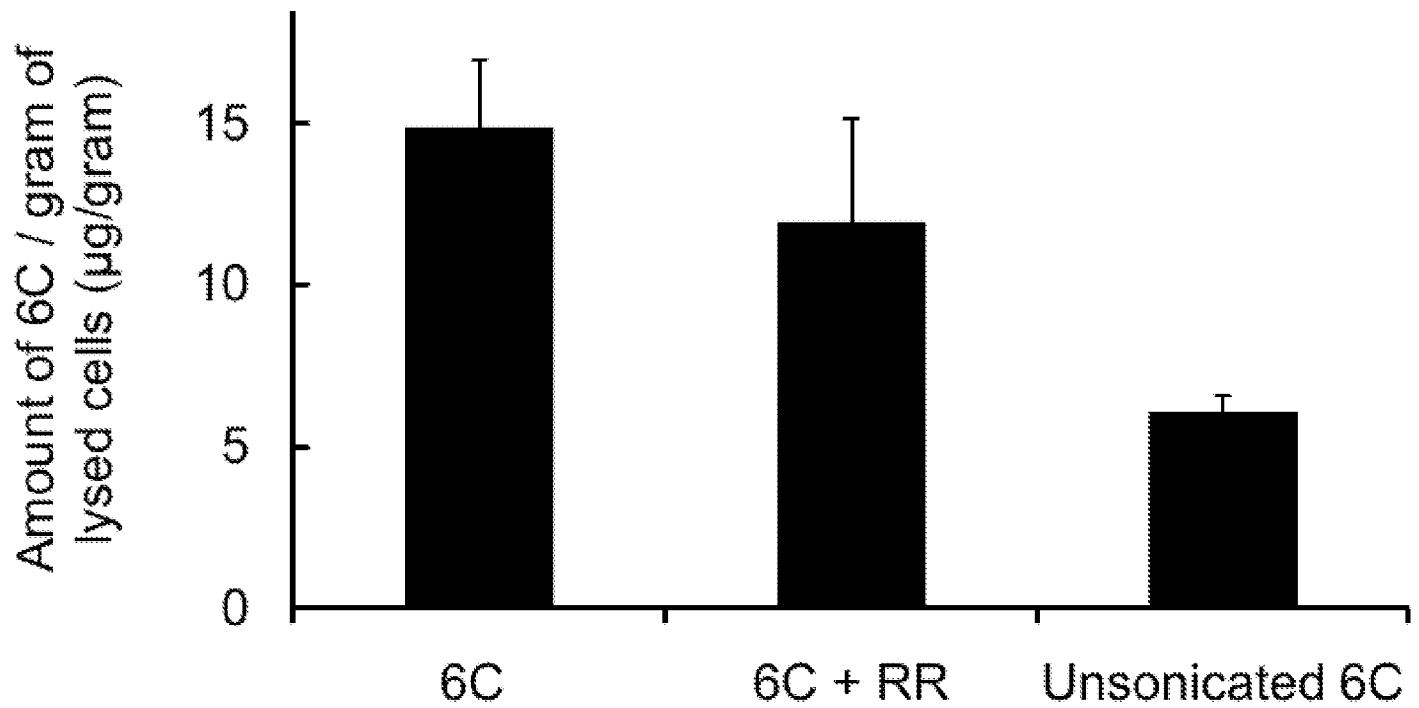


Fig. 5

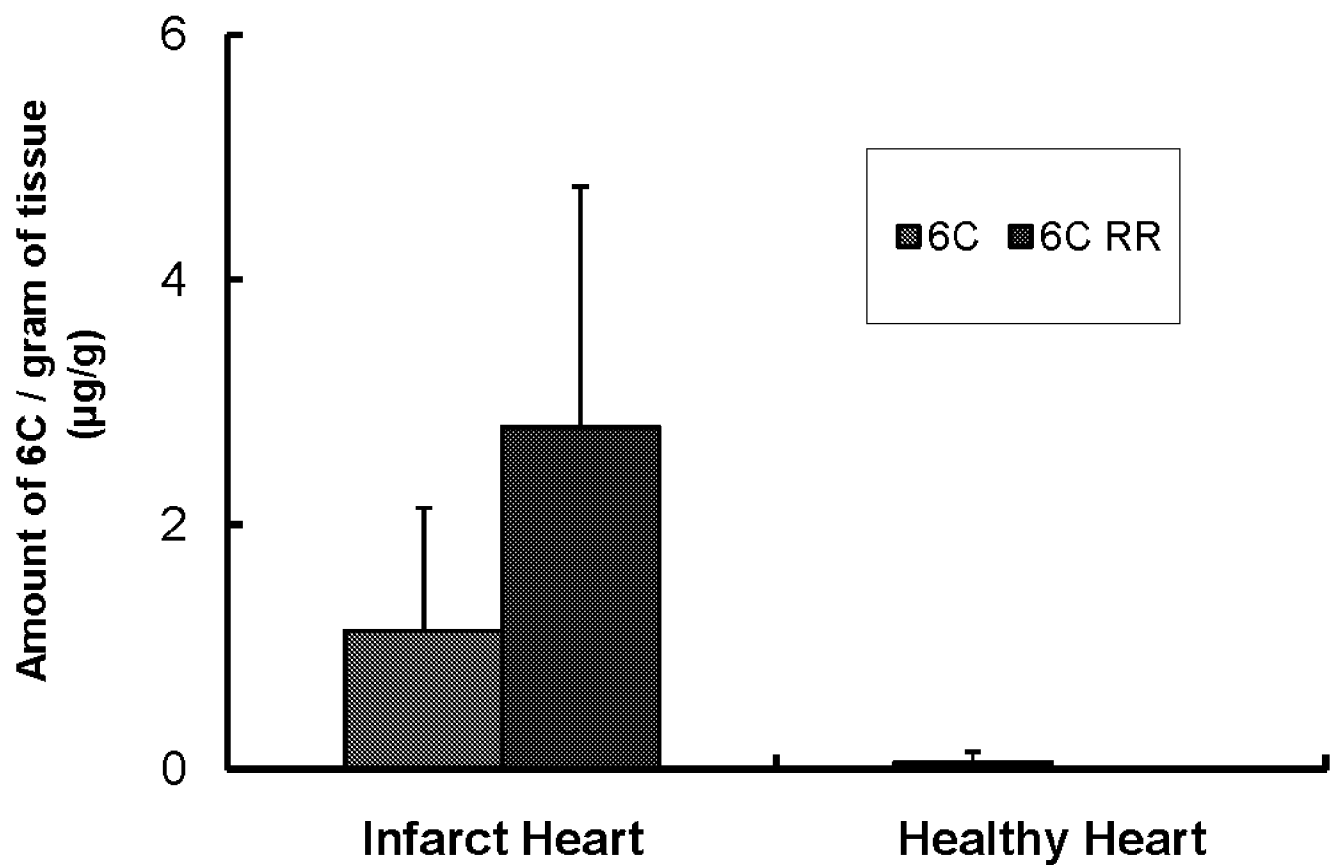


Fig. 6

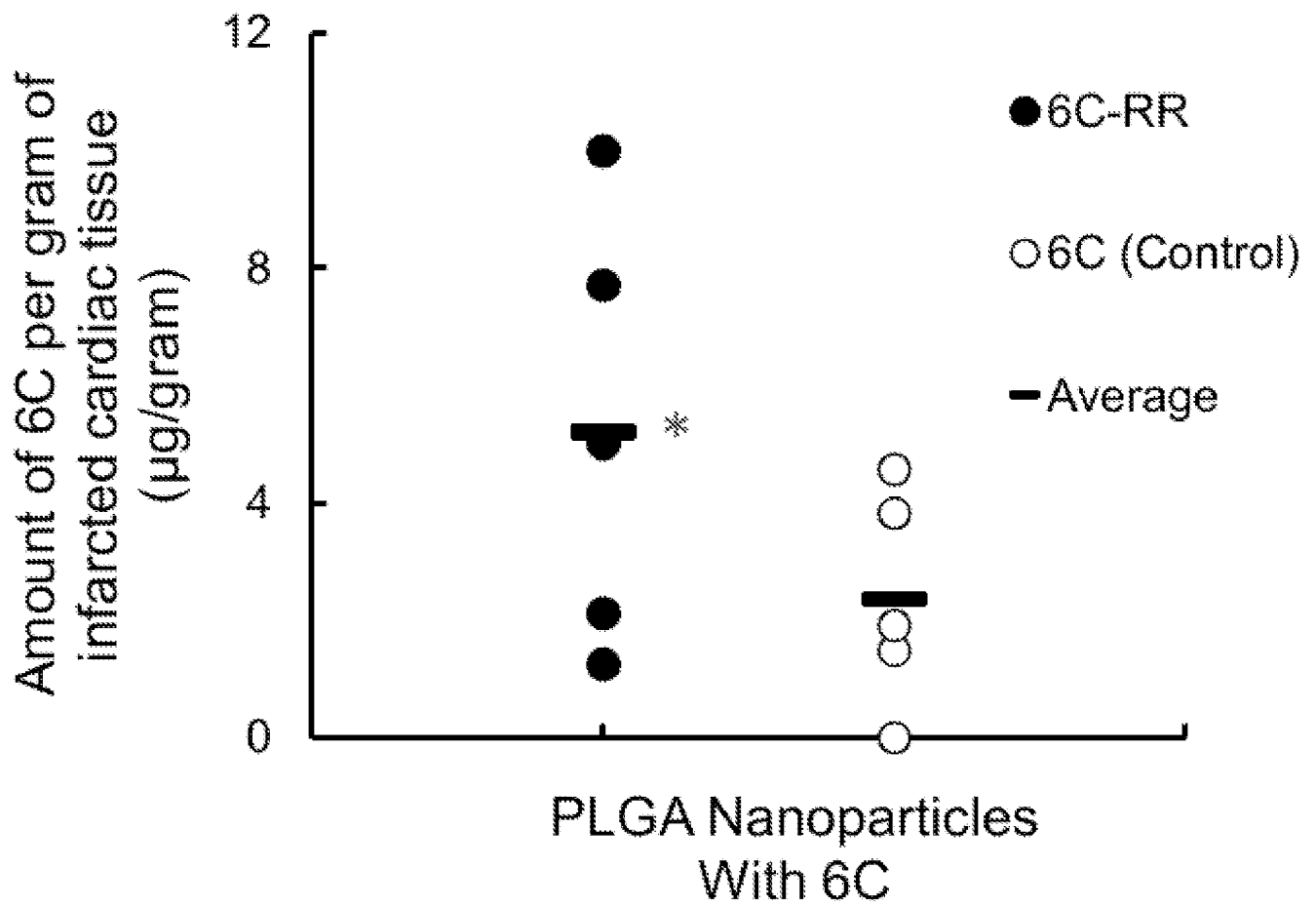


Fig. 7