COMPOSITIONS AND METHODS FOR USING CELLS TO TREAT HEART TISSUE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of priority from International Application No. PCT/US2008/064895, filed on May 27, 2008.

TECHNICAL FIELD

[0002] This document relates to methods and materials involved in obtaining and using cardiac cells. For example, this document relates to methods and materials for providing mammalian heart tissue with cells (e.g., differentiated cardioprogenitor cells or cardiopoietic cells) that incorporate into the heart tissue as functional cardiomyocytes.

BACKGROUND INFORMATION

[0003] Cardiovascular disease is a leading cause of morbidity and mortality worldwide, despite advances in patient management. In contrast to tissues with high reparative capacity, heart tissue is vulnerable to irreparable damage. Cell-based regenerative cardiovascular medicine is, therefore, being pursued in the clinical setting.

[0004] The recent advent of stem cell biology extends the scope of current models of practice from traditional palliation towards curative repair. Typically, clinical experience has been based on adult stem cells recruited from autologous sources and delivered in an unaltered state. First generation biologics are naïve human stem cells, identified as readily accessible cytotypes. It has been shown that particular individuals improve on delivery of naïve human stem cells.

DEFINITIONS

[0005] Within the frame of the present document, and unless indicated to the contrary, the terms designated below between quotes have the following definitions.

[0006] The term ‘hMSCs’ means human mesenchymal stem cells.

[0007] The 'cardio-generative potential' of a cell designates the ability of this cell to succeed to generate cardiac cells for instance myocardium, when injected into an infracted heart.

[0008] 'Cardiopoietic cells' (CP) are cells engaged in the way of differentiation from a non-differentiated cell. A 'cardiopoietic cell' exhibits a cardiac differentiation defined by nuclear translocation of the early cardiac transcription factor Nkx2.5 and the late cardiac transcription factor MEF2C (Behfar et al. Derivation of a cardiopoietic population from human mesenchymal stem yields progeny, Nature Clinical Practice, Cardiovascular Medicine, March 2006 vol. 3 supplement 1, pages S78 – S82). Nuclear translocation of cardiac transcription factor GATA4 can be observed. Cardiopoietic cells can lack sarcomeres and can lack expression of sarcomeric proteins. A cardiopoietic cell keeps the capacity to divide itself. Cardiopoietic cells are also called 'cardiomyocyte precursors' or 'cardiomyocyte progenitor cells' because they may differentiate into cardiomyocytes. In the context of the present document, cardiopoietic cells may be derived from human adult mesenchymal stem cells (hMSCs). 'CP-hMSCs' designates such cardiopoietic cells derived from human adult mesenchymal stem cells.

[0009] A 'cocktail' or 'cardiogenic cocktail' designates a composition containing at least two cardiogenic substances.

[0010] A 'cardiogenic substance' is a substance which improves the cardio-generative potential of a cell.

[0011] A 'cocktail-guided cell' or 'cell guided towards cardiopoiesis' is a cell which has been put into contact with a cocktail and further enters into differentiation.

[0012] The 'differentiation' is the process by which a less specialized cell becomes a more specialized cell.

[0013] The 'ejection fraction' means the fraction of blood pumped out during a heart beat. Without a qualifier, the term ejection fraction refers specifically to that of the left ventricle (left ventricular ejection fraction or LVEF).

[0014] The 'change of ejection fraction' means the difference between ejection fraction of the heart of an animal treated with cells injected into its infarcted heart, measured after a given time and the ejection fraction measured prior to injection.

[0015] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

SUMMARY

[0016] The invention relates to compositions comprising TGFβ-1, BMP4, α-thrombin, a compound selected from the group consisting of Cardiotrophin and IL-6, and a compound selected from the group consisting of Cardiogenol C and retinoic acid. In a preferred embodiment, the compositions of the invention comprise TGFβ-1, BMP4, α-thrombin, Cardiotrophin and Cardiogenol C. The compositions of the invention may comprise at least one compound selected from the group consisting of FGF-2, IGF-1, Activin-A, TNF-α, FGF-4, LIF, VEGF-A and combinations thereof. They may also comprise FGF-2, IGF-1 and Activin-A. Other preferred compositions of the invention comprises Activin-A, FGF-2, IL-6, IGF-1 and retinoic acid. According to an alternative embodiment, the compositions of the invention can lack at least one compound chosen in the group consisting of TNF-α, FGF-4, LIF, and VEGF-A.

[0017] When one of the following compounds is present in a composition of the invention, it may be present in an amount of between 1 and 5 ng of said TGFβ-1 per ml, between 1 and 10 ng of said BMP4 per ml, between 0.5 and 5 ng of said Cardiotrophin per ml, between 0.5 and 5 units of said α-thrombin per ml, and between 50 and 500 nM of said Cardiogenol C, between 1 and 10 ng of said FGF-2 per ml, between 10 and 100 ng of said IGF-1 per ml, between 1 and 50 ng of said Activin-A per ml, between 1 and 50 ng of said TNF-α per ml, between 1 and 20 ng of said FGF-4 per ml, between 10 and 100 ng of said IL-6 per ml, between 1 and 10 units of said LIF per ml, between 1 and 50 ng of said VEGF-A per ml, between 0.1 and 1.0 μM of said retinoic acid per ml.

[0018] Preferred compositions according to the invention comprise recombinant TGFβ-1(2.5 ng/ml), BMP4 (5 ng/ml), Cardiotrophin (1ng/ml), Cardiogenol C (100 μM), used in a combinatorial fashion. Particularly preferred compositions of the invention comprises such compounds and further comprise α-thrombin, (1 U/ml), FGF-2 (10 ng/ml), IGF-1 (50 ng/ml) and Activin-A (5 ng/ml).

[0019] Other preferred compositions of the invention comprise recombinant TGFβ-1 (2.5 ng/ml), BMP4 (5 ng/ml), Activin-A (5 ng/ml), FGF-2 (10 ng/ml), IL-6 (100 ng/ml), Factor-IIa (hα-thrombin, 1 U/ml), IGF-1 (50 ng/ml), and retinoic acid (1 μM) used in a combinatorial fashion.

[0020] Preferably, the compositions of the invention are comprised in a medium selected from the group consisting of media containing of foetal calf serum, human serum, platelet lysate, and mixtures thereof.

[0021] The invention also relates to a method for obtaining from initial cells differentiated cells expressing an elevated level of at least one of the mRNAs selected from the group consisting of MEF2c mRNA, MESP-1 mRNA, Tbx-5 mRNA, GATA4 mRNA , Flk-1 mRNA, GATA6 mRNA, Fog-1 mRNA, and combinations thereof, and/or have at least one polypeptide selected from the group consisting of Nkx2.5 polypeptides, MEF2C polypeptides, Tbx-5 polypeptides, FOG-2 polypeptides, GATA-4 polypeptides MESP-1 polypeptides, and combinations thereof, wherein said at least one polypeptide is associated with the nuclei of said differentiated cells, wherein said method comprises culturing initial cells in the presence of a composition according to the invention In such methods, the differentiated cells express preferably an elevated level of MEF2c mRNA and MESP-1 mRNA.

[0022] In a preferred embodiment of the invention, the initial cells are mesenchymal stem cells. Such cells can be bone marrow-derived stem cells. They can express CD90, CD105, CD133, CD166, CD29, and CD44 on the cell surface and do not express CD14, CD34, and CD45 on the cell surface.

[0023] Most preferably, the differentiated cells are cardiopoietic cells.

[0024] Another aspect of the invention is a method for delivering differentiated cells to a mammal, wherein said method comprises:

(a) determining that a sample of cells from a population of differentiated cells comprises cells that express an elevated level of at least one of the mRNA selected from the group consisting of MEF2c mRNA, MESP-1 mRNA, Tbx-5 mRNA, GATA4 mRNA , Flk-1 mRNA, GATA6 mRNA, Fog-1 mRNA, and combinations thereof, and/or have at least one polypeptide selected from the group consisting of Nkx2.5 polypeptides, MEF2C polypeptides, Tbx-5 polypeptides, MESP-1 polypeptides, GATA-4 polypeptides, FOG-2 polypeptides, and combinations thereof, wherein said polypeptide is associated with the nuclei of said differentiated cells, and

(b) administering cells from said population of differentiated cells to said mammal. Said population of differentiated cells can be obtained from said original cells cultured in the presence of any of said compositions according to the invention. In a particular embodiment, said step (a) may comprise using a reverse transcription polymerase chain reaction or using immunocytochemistry. Said administering step comprises may comprise administering said cells via an administration selected from the group consisting of systemic, intracardiac, and intracoronary administrations.

[0025] Another aspect of the invention is a method for providing heart tissue with cardiomyocytes, wherein said method comprises administering, to said heart tissue, said differentiated cells of claims obtainable by contact with a composition according to the invention.

[0026] Details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and from the claims.

DETAILED DESCRIPTION

[0027] This document provides methods and materials related to cardiac cells (e.g., differentiated cardioprogenitor cells). For example, this document provides cells having the ability to incorporate into heart tissue as functional cardiomyocytes, methods for making such cells, compositions for making such cells, and methods for determining whether or not a population of cells (e.g., differentiated cardioprogenitor cells) contains cells having the ability to incorporate into heart tissue as functional cardiomyocytes. This document also provides methods and materials for providing heart tissue (e.g., human heart tissue) with functional cardiomyocytes.

[0028] The differentiated cardioprogenitor cells provided herein can be from any species including, without limitation, humans, monkeys, horses, dogs, cats, rats, or mice. For example, differentiated cardioprogenitor cells can be mammalian (e.g., human) differentiated cardioprogenitor cells.

[0029] In some cases, differentiated cardioprogenitor cells provided herein have the ability to incorporate into heart tissue as functional cardiomyocytes.

[0030] Any appropriate method can be used to obtain differentiated cardioprogenitor cells. For example, differentiated cardioprogenitor cells can be derived from stem cells such as mammalian (e.g., human) stem cells.

[0031] In some cases, differentiated cardioprogenitor cells can be derived from embryonic stem cells. In some cases, differentiated cardioprogenitor cells can be derived from mesenchymal stem cells. Mesenchymal stem cells can be obtained from any source. For example, mesenchymal stem cells can be obtained from mammalian (e.g., human) tissue such as bone marrow and trabecular bone. Mesenchymal stem cells can be cultured *in vitro*. For example, mesenchymal stem cells can be expanded in number *in vitro*. Mesenchymal stem cells can express or not express a polypeptide marker on the cell surface. For example, mesenchymal stem cells can express CD133, CD90, CD105, CD166, CD29, and CD44 on the cell surface and not express CD14, CD34, and CD45 on the cell surface.

[0032] Any appropriate method can be used to derive differentiated cardioprogenitor cells from stem cells (e.g., mesenchymal stem cells). For example, differentiated cardioprogenitor cells can be derived from mesenchymal stem cells by incubating the mesenchymal stem cells with a composition (e.g., culture media). The composition can be any appropriate composition containing one or more factors. The factors can be any type of factors such as polypeptides, steroids, hormones, and small molecules. Examples of such factors include, without limitation, TGFβ, BMP, FGF-2, IGF-1, Activin-A, Cardiotrophin, α-thrombin, and Cardiogenol C.

[0033] In one embodiment, media containing TGFβ, BMP, Cardiotrophin, α-thrombin, and Cardiogenol C can be used to obtain differentiated cardioprogenitor cells from stem cells (e.g., mesenchymal stem cells). In such cases, FGF-2, IGF-1, Activin-A, or a combination thereof can be added to the medium after an initial culture period (e.g., one or two days) with medium containing TGFβ, BMP, Cardiotrophin, α-thrombin, and Cardiogenol C.

[0034] TGFβ can be any polypeptide having TGFβ activity, such as human TGFβ. For example, TGFβ can be recombinant TGFβ or synthetic TGFβ. In one embodiment, TGFβ can be TGFβ-1. Any appropriate concentration of TGFβ can be used. For example, between 1 and 10 ng of TGF-β per ml (e.g., about 2.5 ng of TGFβ1 per ml) can be used.

[0035] BMP can be any polypeptide having BMP activity, such as human BMP. For example, BMP can be recombinant BMP or synthetic BMP. In one embodiment, BMP can be BMP4. Any concentration of BMP can be used. For example, between 1 and 20 ng of BMP per ml (e.g., about 5 ng of BMP4 per ml) can be used.

[0036] FGF-2 can be any polypeptide having FGF-2 activity, such as human FGF-2. For example, FGF-2 can be recombinant FGF-2 or synthetic FGF-2. Any concentration of FGF-2 can be used. For example, between 1 and 20 ng of FGF-2 per ml (e.g., about 5 ng of FGF-2 per ml) can be used.

[0037] IGF-1 can be any polypeptide having IGF-1 activity, such as human IGF-1. For example, IGF-1 can be recombinant IGF-1 or synthetic IGF-1. Any concentration of IGF-1 can be used. For example, between 10 and 100 ng of IGF-1 per ml (e.g., about 50 ng of IGF-1 per ml) can be used.

[0038] Activin-A can be any polypeptide having Activin-A activity, such as human Activin-A. For example, Activin-A can be recombinant Activin-A or synthetic Activin-A. Any concentration of Activin-A can be used. For example, between 1 and 50 ng of Activin-A per ml (e.g., about 10 ng of Activin-A per ml) can be used.

[0039] α-Thrombin can be any polypeptide having α-thrombin activity, such as human α-thrombin. For example, α-thrombin can be recombinant α-thrombin or synthetic α-thrombin. Any concentration of α-thrombin can be used. For example, between 0.5 and 10 units of α-thrombin per ml (e.g., about 1 unit of α-thrombin per ml) can be used.

[0040] Cardiotrophin can be any polypeptide having Cardiotrophin activity, such as human Cardiotrophin-1. For example, Cardiotrophin can be recombinant Cardiotrophin or synthetic Cardiotrophin. Any concentration of Cardiotrophin can be used. For example, between 0.5 and 10 ng of Cardiotrophin per ml (e.g., about 1 ng of Cardiotrophin-1 per ml) can be used.

[0041] IL-6 can be any polypeptide having IL-6 activity, such as human IL-6. For example, IL-6 can be recombinant IL-6 or synthetic IL-6. Any concentration of IL-6 can be used. For example, between 100 and 200 ng of IL-6 per ml can be used.

[0042] Any concentration of Cardiogenol C or a pharmaceutically acceptable salt thereof (e.g., Cardiogenol C hydrochloride) can be used. For example, between 10 and 1000 nM of Cardiogenol C (e.g., about 100 nM of Cardiogenol C) can be used.

[0043] Retinoic acid can be any molecule having retinoic acid activity, such as synthetic retinoic acid, natural retinoic acid, a vitamin A metabolite, a natural derivative of vitamin A, or a synthetic derivative of vitamin A. Any concentration of retinoic acid can be used. For example, between 1 x 10^{-6} and 2 x 10^{-6} μM of retinoic acid can be used.

[0044] In some cases, serum-containing or serum-free media supplemented with TGFβ-1 (e.g., 2.5 ng/ml), BMP4 (e.g., 5 ng/ml), FGF-2 (e.g., 5 ng/ml), IGF-1 (e.g., 50 ng/ml), Activin-A (e.g., 10 ng/ml), Cardiotrophin (e.g., 1 ng/ml), α-thrombin (e.g., 1 Unit/ml), and Cardiogenol C (e.g., 100 nM) can be used to obtain differentiated cardioprogenitor cells from stem cells (e.g., mesenchymal stem cells). In some cases, the media (e.g., serum-containing or serum-free media) can contain platelet lysate (e.g., a human platelet lysate)

[0045] In some cases, the composition used to obtain differentiated cardioprogenitor cells from mesenchymal stem cells can contain additional optional factors such as TNF-α, LIF, and VEGF-A.

[0046] TNF-α can be any polypeptide having TNF-α activity, such as human TNF-α. For example, TNF-α can be recombinant TNF-α or synthetic TNF-α. Any concentration of TNF-α can be used. For example, between 5 and 50 ng of TNF-α per ml can be used.

[0047] LIF can be any polypeptide having LIF activity, such as human LIF. For example, LIF can be recombinant LIF or synthetic LIF. Any concentration of LIF can be used. For example, between 2.5 and 100 ng of LIF per ml can be used.

[0048] VEGF-A can be any polypeptide having VEGF-A activity, such as human VEGF-A. For example, VEGF-A can be recombinant VEGF-A or synthetic VEGF-A. Any concentration of VEGF-A can be used. For example, between 5 and 200 ng of VEGF-A per ml can be used.

[0049] A composition provided herein can contain any combination of factors. For example, a composition provided herein can contain TGFβ-1, BMP4, Activin-A, Cardiotrophin, α-thrombin, and Cardiogenol C. In some cases, a composition provided herein can contain TGFβ-1, BMP4, FGF-2, IGF-1, Cardiotrophin, α-thrombin, and Cardiogenol C. In some cases, a composition provided herein can contain TGFβ-1, BMP4, FGF-2, IGF-1, Cardiotrophin, α-thrombin, and Cardiogenol C. In some cases, a composition provided herein can lack TNF-α, IL-6, LIF, VEGF-A, retinoic acid, or any combination thereof (e.g., IL-6, LIF, VEGF-A, and retinoic acid; LIF, VEGF-A, and retinoic acid; or TNF-α, IL-6, LIF, and VEGF-A).

[0050] A composition provided herein can be prepared using any appropriate method. For example, a composition provided herein can be prepared using commercially available factors. In some cases, a composition provided herein can be prepared to contain cells lysates (e.g., a platelet lysate) or conditioned media from cells such as cardiomyocyte cells or TNF-α-stimulated endodermal cells. For example, a composition provided herein can be prepared using a platelet lysate supplemented with commercially available factors. In some cases, a composition provided herein can be prepared using factors isolated from conditioned medium. In some cases, the factors can be dissolved in media such as cell culture media that does or does not contain serum.

[0051] Any appropriate method can be used to incubate stem cells (e.g., mesenchymal stem cells) with a composition provided herein to obtain differentiated cardioprogenitor cells having the ability to incorporate into heart tissue as functional cardiomyocytes. For example, mesenchymal stem cells can be incubated with a composition provided herein for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, or 50 days. In some cases, a composition provided herein and used to incubate mesenchymal stem cells can be replaced every day or every 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, or 50 days.

[0052] In some cases, mesenchymal stem cells can be incubated with a composition provided herein in the presence or absence of serum. Any appropriate cell density can be used when incubating stem cells with a composition provided herein. For example, between about 1000 and 2000 mesenchymal stem cells per cm^2 (e.g., between about 1500-2000 cells/cm^2) can be incubated with a composition provided herein to obtain differentiated cardioprogenitor cells.

[0053] Once stem cells (e.g., mesenchymal stem cells) have been incubated with a composition provided herein or otherwise treated with differentiation factors, the state of differentiation can be monitored to determine whether or not the stem cells differentiated into differentiated cardioprogenitor cells having the ability to incorporate into heart tissue as functional cardiomyocytes. For example, a sample of cells can be collected and assessed using techniques such as Western blotting, fluorescence-activated cell sorting (FACS), immunostaining, laser confocal microscopy, and reverse transcription polymerase chain reaction (RT-PCR) techniques (e.g., quantitative RT-PCR). In some cases, cells found to express an elevated level of MEF2c, MESP-1, Tbx-5, Nkx2.5, GATA6, Flk-1, Fog 1 and Fog 2 polypeptides or mRNA can be selected for administration into a mammal to treat heart tissue.

[0054] As described herein, differentiated cardioprogenitor cells derived from mesenchymal stem cells cultured with a platelet lysate containing TGFβ-1, BMP4, FGF-2, IGF-1, Activin-A, Cardiotrophin, α-thrombin, and Cardiogenol C exhibited a 2 to 5-fold increase in MEF2c mRNA, MESP-1 mRNA, Tbx-5 mRNA, GATA6 mRNA, Flk-1 or Fog 1 mRNA levels as compared to the levels observed with pre-treated mesenchymal stem cells. These differentiated cardioprogenitor cells also exhibited the ability to incorporate into heart tissue as functional cardiomyocytes when injected intramyocardially, subcutaneously, or intravascularly with heart pump function improvement directly correlated with structural repair in both ischemic and non-ischemic settings. Functional benefit was documented both echocardiographically *in vivo*, and histologically on autopsy through staining of human specific proteins. Also as described herein, differentiated cardioprogenitor cells derived from mesenchymal stem cells cultured with serum containing TGFβ-1, BMP4, FGF-2, IGF-1, Activin-A, Cardiotrophin, α-thrombin, and Cardiogenol C exhibited a 5 to 10-fold increase in MEF2c mRNA, MESP-1 mRNA, and Tbx-5 mRNA levels as compared to the levels observed with pre-treated mesenchymal stem cells.

[0055] These differentiated cardioprogenitor cells also exhibited the ability to incorporate into heart tissue as functional cardiomyocytes when injected intramyocardially (e.g., through endocardial or epicardial routes), into the coronary arteries, infused in the heart, or administered systemically (e.g., subcutaneously), with heart pump function improvement directly correlated with structural repair in both ischemic and non-ischemic settings. Functional benefit was documented by cardiac ultrasound *in vivo*, and by microscopic analysis on autopsy through staining of human specific proteins. Thus, release criteria such as elevated polypeptide or mRNA levels of MEF2c, MESP-1, Tbx-5, GATA6, Flk-1, Fog 1, FOG 2, or combinations thereof can be used to evaluate cells prior to administration into a mammal.

[0056] The term "elevated level" as used herein with respect to polypeptide or mRNA levels of MEF2c, MESP-1, Tbx-5, GATA6, Flk-1, or Fog (for instance FOG 1 for mRNA, FOG 2 for the polypeptide) within a cell population refers to any level that is greater than a reference level for that polypeptide or mRNA.

[0057] The term "reference level" as used herein with respect to polypeptide or mRNA levels of MEF2c, MESP-1, Tbx-5, GATA6, Flk-1, or Fog (for instance FOG 1 for mRNA, FOG 2 for the polypeptide) within a cell population refers to the level typically found in pre-treated cells (e.g., pre-treated mesenchymal stem cells). For example, an MEF2c mRNA reference level, an MESP-1 mRNA reference level, a Tbx-5 mRNA reference level, a GATA6 mRNA reference level, and a FOG 1 mRNA reference level can be the average level of MEF2c, MESP-1, Tbx-5, GATA6, Flk-1 and FOG 1 mRNA, respectively, that is present in a random sampling of mesenchymal stem cells not treated with a composition provided herein or otherwise treated with differentiation factors. It will be appreciated that levels from comparable samples are used when determining whether or not a particular level is an elevated level.

[0058] Elevated polypeptide and/or mRNA levels of MEF2c, MESP-1, Tbx-5, GATA 4, GATA6, Flk-1, Fog 2 or FOG 1 can be any level provided that the level is greater than a corresponding reference level.

[0059] For example, an elevated level of Tbx-5 mRNA can be 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more fold greater than the reference level Tbx-5 mRNA observed in untreated mesenchymal stem cells. It is noted that a reference level can be any amount. For example, a reference level for Tbx-5 mRNA can be zero. In this case, any level of Tbx-5 mRNA greater than zero would be an elevated level.

[0060] In some cases, identification criteria can include microscopic analysis of cells prior to administration into a mammal. Such microscopic analysis can include assessing the cells for transcription factor polypeptides associated with the nucleus. For example, cells appropriate for release into a mammal can be assessed for the presence of Nkx2.5, MEF2c, GATA4, MESP-1, FOG 2, Tbx-5, or any combination thereof associated with the nucleus before being released into the mammal.

[0061] Any appropriate method can be used to provide heart tissue with differentiated cardioprogenitor cells having the ability to incorporate into heart tissue as functional cardiomyocytes. For example, differentiated cardioprogenitor cells can be injected intramyocardially (e.g., through endocardial or epicardial routes), into the coronary arteries, infused in the heart, or administered systemically (e.g., subcutaneously).

[0062] Any heart tissue can be provided with differentiated cardioprogenitor cells. For example, mammalian (e.g., human) heart tissue can be provided with differentiated cardioprogenitor cells. In some cases, heart tissue that has suffered from ischemic cardiomyopathy, myocardial infarction, or heart failure can be provided with differentiated cardioprogenitor cells.

[0063] Any type of differentiated cardioprogenitor cells can be administered to heart tissue. For example, autologous or heterologous differentiated cardioprogenitor cells can be administered to heart tissue. In some cases, stem cells (e.g., mesenchymal stem cells) that were incubated with a composition provided herein can be administered to heart tissue.

[0064] The stem cells can be incubated with a composition provided herein for any length of time before being administered to heart tissue. For example, the stem cells can be incubated with a composition provided herein for 6 to 24 hours (e.g., 8, 10, 12, 18, or 22 hours) or for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, or 50 days before being administered to heart tissue. In some cases, stem cells that were incubated with a composition provided herein can be administered to heart tissue together with a composition provided herein.

[0065] The stem cells can be incubated with a composition provided herein for any length of time before being administered to heart tissue together with a composition provided herein. For example, the stem cells can be incubated with a composition provided herein for 6 to 24 hours (e.g., 8, 10, 12, 18, or 22 hours) or for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, or 50 days before being administered to heart tissue together with a composition provided herein.

[0066] In some cases, differentiated cardioprogenitor cells can be assessed to determine whether or not they meet particular release criteria prior to being administered to a mammal. For example, differentiated cardioprogenitor cells can be assessed using RT-PCR to confirm that the differentiated cardioprogenitor cells express an elevated polypeptide or mRNA level of MEF2c, MESP-1, Tbx-5, GATA6, Flk-1, Fog, (FOG 1 for mRNA, FOG 2 for the polypeptide) or combinations thereof before being administered into a mammal.

[0067] The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0068] Fig. 1 represents the change of ejection fraction (ΔEF) in %, considered before and after treatment with naïve human mesenchymal stem cells (hMSCs) derived from bone narrow of 11 patients with coronary artery disease.

[0069] Fig. 2 obtained on confocal microscopy after immunostaining with DAPI, shows protein expression of the cardiac transcription factor content for patient 2 that demonstrated no positive ejection fraction change (left) and for patient 9 (right) that demonstrated a positive ejection fraction change after treatment.

[0070] Fig. 3 shows the mRNA expression of two significant cardiac transcription factors mRNA in the hMSCs of the eleven patients.

[0071] Fig. 4 shows the mRNA expression, in arbitrary units (A.U.) of cardiac transcription factors for respectively Nkx2.5 mRNA, GATA-6 mRNA and Fog-1 mRNA, of untreated, naïve hMSCs (left) and on CP-hMSCs, treated with a cardiogenic cocktail (right).

[0072] Fig. 5 shows images obtained on confocal microscopy showing nucleartranslocation of Nkx2.5, MEF2C, FOG-2 and GATA4 polypeptides in CP-hMSCs treated with a cardiogenic cocktail (right), compared with naïve hMSCs (left).

[0073] Fig. 6 illustrates the progressive conversion (at days D0, D5, D15, and day D20) from naïve hMSCs to 'cocktail-guided' CP-hMSCs and eventually cardiomyocytes (CM).

[0074] Fig. 7 shows the transition electron microscopy ultrastructure of naïve hMSC and cocktail-guided cardiomyocyte.

[0075] Fig. 8 shows a cocktail-guided cardiomyocyte in light microscopy.

[0076] Fig. 9 represents, on the first graph on the left, the Tbx-5 mRNA expression (in AU) for naïve hMSC and CP-hMSC from each patient of Fig. 1, the results for the naïve cells being on the right and the one for the CP cells being on the left of the histogram. The second graph in the middle presents MEF2C mRNA expression, and the third graph on the right presents MESP-1 mRNA expression. The average values for the naïve cells from all patients and the one for the CP cells for all patients are given in the background of the histogram.

[0077] Fig. 10 is a graph which represents the ejection fraction change (ΔEjection Fraction) after treatment of heart with different quantities of naïve hMSC (right part of histogram for each of twelve patients) versus treatment of hearts with CP-hMSCs, i.e. cocktail-guided hMSC (CP-hMSC).

[0078] Fig. 11 represents the echocardiography of infarcted hearts untreated (left) and treated with cardiopoietic cells (right), which shows a far better anterior wall reanimation upon treatment with CP-hMSCs.

[0079] Fig. 12 is a graph similar to Fig. 5 showing the ejection fraction change (ΔEF) after injection of naïve (on the left) and cardiopoietic cells (on the right) into infracted myocardium. Sham is injection without cells.

[0080] Fig. 13 shows murine infarcted hearts treated with naïve hMSC and CP-hMSCs six months following treatment initiation. Aneurysms and scar, which remained uncorrected in naïve hMSCs treated hearts, were resolved with CP-hMSCs treatment that induced re-muscularization.

[0081] Fig. 14 shows that confocal resolution revealed, in the cardiopoietic hMSC treated murine myocardium, widespread presence of human-derived cells with positive staining for h-ALU-DNA sequences specific to the human species validated with human-specific lamin immunostaining, all of which were absent in infarcted controls.

[0082] Fig. 15 shows that cardiomyocytes of human origin were tracked by co-localization of human cardiac troponin-I and α-actinin in cardiopoietic hMSC-treated hearts. Cardiomyocytes of human origin were absent from naïve hMSC-treated hearts. Quantification within infarcted anterior walls revealed 3±2% and 25±5% of myocardial nuclei in naïve *versus* CP-hMSC-treated hearts, implying enhanced engraftment with cardiopoietic hMSC treatment.

[0083] Figure 16 contains photographs of naïve and CP hMSC stained for human troponin, ventricular myosin light chain mlC2V, and DAPI. Ventricular cell phenotype was corroborated with counter staining of human-troponin positive cells with ventricular myosin light chain mlC2V immunostaining in repaired anterior wall, as shown in Fig. 15 or resolving scar as shown in Fig. 17.

Figure 17 is a photograph of a remnant scar stained for human troponin, ventricular myosin light chain mlC2V, and DAPI.

[0084] Fig. 18 contains photographs of CP-hMSCs-treated regenerating myocardium demonstrating angiogenesis distal to the occluded coronary vessel.

[0085] Fig. 19 demonstrates CP-hMSCs contribution to neo-vascularization via expression of human PECAM-1 (CD-31) within the myocardial vasculature.

[0086] Fig. 20 is a graph plotting Δ ejection fraction relative to sham (%) versus time (months) following cell delivery. The long-term impact of CP-hMSCs treatment was tracked for more than one 1-year, or one third of murine lifespan which would translate into 25-years of human life. Relative to sham, treatment with naïve hMSC showed a 5% and 2.5% ejection fraction effect at 6 and 12 months, respectively. In contrast, cardiopoietic hMSC treated infarcted mice demonstrated significant ejection fraction improvement of 25% at 6 and 12 months relative to sham.

[0087] Fig. 21 is a graph plotting Δ ejection fraction (%) versus time (months) post cell transplantation. The infarcted cohort was stratified to evaluate efficacy in subgroups with documented overt heart failure (ejection fraction <45%) at the time of intervention. Despite equivalent pre-treatment ejection fraction at 35%, only cardiopoietic hMSC treatment improved absolute ejection fraction by 10% at 6 and 12-months, in contrast to a 5% decline in ejection fraction in the naïve hMSC-treated cohort.

[0088] Fig. 22 is a bar graph plotting survivorship (%) for the indicated subgroups of mice. In overt heart failure subgroups at 400 days follow-up, no survivors were present in the sham and mortality of >50% was recorded with naïve hMSC treatment. In contrast, a >80% survival was attained with cardiopoietic hMSC treatment.

[0089] Fig. 23 illustrates the safety of treatment with CP-hMSCs, determined by pathological examination and electrocardiography.

EXAMPLES

[0090] Patients undergoing coronary artery bypass for ischemic heart disease were randomly selected for bone marrow harvest. They provided informed consent, and study protocols were approved by pertinent Institutional Ethics Committee and Institutional Animal Care and Use Committee. Is worth noting that no injections were made to patients but to mice.

Example 1

[0091] Mesenchymal stem cells were derived from human bone marrow withdrawn from the posterior iliac crest of the pelvic bone of 18- to 45-year-old healthy individuals (Cambrex, East Rutherford, New Jersey). Based on flow cytometry analysis, the mesenchymal stem cells expressed CD90, CD133, CD105, CD166, CD29, and CD44, and did not express CD14, CD34, and CD45.

[0092] Human bone marrow-derived mesenchymal stem cells were cultured in either platelet lysate or serum supplemented with TGFβ-1 (2.5 ng/ml), BMP4 (5 ng/ml), FGF-2 (5 ng/ml), IGF-1 (50 ng/ml), Activin-A (10 ng/ml), Cardiotrophin (1 ng/ml), α-thrombin (1 Unit/ml), and Cardiogenol C (100 nM). After 4-10 days in the platelet lysate-containing culture at a density of about 1000-2000 cells per cm^2, the cells were found to express 2-5-fold more MEF2c mRNA, MESP-1 mRNA, Tbx-5 mRNA, GATA6 mRNA, Flk-1 or FOG 1 mRNA than untreated mesenchymal stem cells.

[0093] After 5-15 days in the serum-containing culture at a density of about 1000-2000 cells per cm^2, the cells were found to express 5-10-fold more MEF2c mRNA, MESP-1 mRNA, Tbx-5 mRNA, GATA 4 mRNA, GATA6 mRNA, Flk-1 or FOG 1 mRNA than untreated mesenchymal stem cells.

[0094] The primer pairs used for the RT-PCR analysis were standard primers obtained commercially from Applied Biosystems.

[0095] Results demonstrating that the differentiated cardioprogenitor cells have the ability to incorporate into heart tissue as functional cardiomyocytes were obtained both *in vivo* within the beating heart, and *in vitro* following autopsy. *In vivo*, under isoflurane anesthesia, direct myocardial delivery of cardioprogenitor cells into diseased hearts improved cardiac performance as monitored by echocardiography in the short axis with a two-dimensional M-mode probing in the long axis, Doppler pulse wave analysis, and 12-lead electrocardiography.

[0096] Harvested heart tissue was fixed in 3% paraformaldehyde, sectioned, and subjected to immuno-probing for human cell tracking. New human derived cardiomyocytes and vasculature, with functional improvement and scar resolution, was documented on analysis in mice treated with cardioprogenitor cells fulfilling release criteria (e.g., elevated level of expression of MEF2c mRNA, MESP-1 mRNA, Tbx-5 mRNA, GATA 4 mRNA, GATA6 mRNA, Flk-1 or FOG 1 mRNA), in contrast to absence of benefit with cells that did not pass the release criteria.

[0097] In order to scale-up the production of cardiopoietic cells for autologous injection in patients, an alternative method was considered as immunofluorescence can be time-consuming, qualitative and potentially operator-dependent. One method of choice is real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR). This method gives faster results (within one day) that are operator-independent and quantified relative to a reference standard. In addition, while immunostained samples require one by one fluorescent microscopy evaluation, up to 48 different samples (or conditions) can be tested in duplicate by RT-qPCR using 96-well plates.

[0098] In order to identify suitable markers for RT-QPCR, cardiopoietic cells were derived from bone marrow samples obtained from cardiac patients (n=7). Cells were evaluated by immunofluorescence staining for MEF2C and Nkx2.5. RNA was extracted from these cells and expression of Nkx2.5 and MEF2C measured by real-time quantitative PCR.

[0099] The reference standard consisted of cells from the same batch not cultured in the presence of the cardiogenic cocktail.

[0100] Results were calculated using the double delta-Ct method normalizing the data obtained from treated cells to those from untreated cells.

[0101] MEF2C was identified as suitable marker of cardiopoietic cells by both qPCR and immunofluorescence (nuclear translocation) when compared to naive cells. By contrast, the qualitative change in Nkx2.5 seen at the protein level by immunofluorescence (nuclear translocation) was initially not translated into a quantitative change at the RNA level relative to untreated cells. Genes downstream of Nkx2.5 were then investigated, since induction of their expression would depend on nuclear translocation of Nxk2.5. This led to the identification of MESP-1, Flk-1 and Tbx5 as additional suitable genes for identification by QPCR.

[0102] Human bone marrow aspirates (15-20 ml) were obtained during coronary artery bypass surgery following sternotomy. Bone marrow was cryostored in a DMSO-based serum-free freezing solution. Mesenchymal stem cells were recruited by platting of raw bone marrow on plastic dishes with a wash at 12 h selecting adhesive cells with identity confirmed by Fluorescence-Activated Cell Sorting (FACS) analysis using the $CD34^-/CD45^-/CD133^+$ marker panel. Cells were cultured at 37° C in DMEM supplemented with 5% human platelet lysate (Mayo Clinic Blood Bank, Rochester, MN).

[0103] Myocardial infarction was performed in nude, immunocompromised mice (Harlan, Indianapolis, IN). Following a blinded design, one month post-infarction a total of 600,000 naïve or cardiopoiesis guided hMSC, suspended in 12.5 µl of propagation medium, was injected under microscopic visualization in five epicardial sites on the anterior wall of the left ventricle. Sham underwent the same surgical procedure without cell injection. Myocardial injection of bone marrow hMSC into this chronic infarction model demonstrated heterogeneity in outcome with transplantation of cells from only two out of the eleven studied individuals improving ejection fraction on echocardiography.

[0104] Patients 3 and 9 were identified as individuals with a high cardio-generative potential. It was first observed from Fig. 1 that the change of ejection fraction in mice (n=3) treated with hMSC from each patients 3 and 9 was significantly positive, whereas the change for each other patient was not.

[0105] The protein expression of cardiac transcription factors was observed in hMSC on confocal microscopy, as shown in Fig. 2. Bar corresponds to 20 µm representative for all panels.

[0106] Immunostaining was performed with antibodies specific for MEF2C (1:400, Cell Signaling Technologies, Danvers, MA), Nkx2.5 (1:150, Santa Cruz Biotechnology Inc., Santa Cruz, CA), GATA4 (Santa Cruz Biotechnology Inc.), Phospho-AKT^{Ser473} (1:100, Cell Signaling Technologies), Tbx5 (1:5000, Abcam, Cambridge, MA), Mesp-1 (1:250, Novus Bio, Littleton, CO), Fog-2 (1:100, Santa Cruz Biotechnology), sarcomeric protein α-actinin (1:500, Sigma-Aldrich) and human-specific Troponin-I (1:100, Abcam), mlC2v (1:500, Synaptic Systems, Gottigen, Germany), Sca-1 (1:100, R&D Systems, Minneapolis, MN), CD-31/PECAM-1 (1:500, Beckman Coulter, Fullerton, CA), α-smooth muscle actin (Abcam), human-specific Troponin-I (1:100, Abcam), human Lamin A/C (1:50, Novacastra, New Castle, UK), and Ki67 (1:500, Abcam) following fixation in 3% paraformaldehyde and permeabilization with 1% Triton X-100, and along with DAPI staining to visualize nuclei on confocal microscopy performed with a LSM 510 Laser scanning confocal microscope (Carl Zeiss Inc., Jena, Germany).

[0107] Early cardiac transcription factors Nkx 2.5, Tbx-5 and MESP1 late cardiac transcription factor MEF2C were observed under staining with DAPI. The results for patient 2 are on the left, the one for patient 9 on the right. The images obtained show that the expression of the cardiac transcription factors is weak for the hMSC from patient 2 and high for the one of patient 9. This corroborates the fact that the hMSC from patient 9 give an efficient therapeutic benefit whereas the hMSC from patient 2 do not. The coloration afforded by DAPI is blue.

[0108] On Fig. 2 the first series of images for Nkx 2.5 show the nuclei of the cells colored DAPI (left) solely blue for the hMSC of patient 2 (left). A weak green colouration corresponding to the presence of Nkx 2.5 in the cytoplasm also appears. The corresponding image for patient 9 (right) shows a higher expression of Nkx 2.5 (green) in the cytoplasm and also in the nuclei of the cells.

[0109] The second series of images show the cardiac transcription factors Tbx-5 (green) and MESP-1 (red) for patient 2, the nuclei of the cells and coloured in blue by the DAPI, no green or red colour is visible, which corresponds to no expression of TbX-5 and MESP-1. For patient 2, the cytoplasms of the cells are coloured in red and the nuclei in green, which corresponds to strong expression of both cardiac transcription factors and to a translocation of Tbx-5 to the nuclei of the cells.

[0110] The third series of images gives results for MEF2C similar to the one for Nkx 2.5.

[0111] Fig. 3 shows the mRNA expression studied in qPCR revealing cardiac transcription factor expression (MEF2C and Tbx-5) for the hMSC of the eleven patients of the study.

[0112] Quantitative polymerase chain reaction (qPCR) was performed using a TaqMan PCR kit with an Applied Biosystems 7,900HT Sequence Detection System (Applied Biosystems, Foster City, CA). TaqMan Gene Expression reactions were incubated in a 96-well plate and run in triplicate. The threshold cycle (C_T) was defined as the fractional cycle number at which fluorescence passes a fixed threshold. TaqMan C_T values were converted into relative fold changes determined using the $2^{-\Delta\Delta C}{}_T$ method, normalized to *GAPDH* (P/N 435,2662-0506003) expression.

[0113] Genes listed in Table 1, which are representative of cardiac transcriptional activity were evaluated.

[0114] Cells were evaluated at the mRNA and protein levels prior to and following a 5-day stimulation with a cardiogenic cocktail comprising human recombinant TGFβ-1 (2.5 ng/ml), BMP4 (5 ng/ml), Cardiotrophin (1 ng/ml), α-thrombin (1 U/ml), and Cardiogenol C (100 μM). Both the MEF2C mRNA and the Tbx-5 mRNA expressions (in arbitrary units AU) are much higher for the hMSC of patients 2 and 9 than for the one of other patients.

Applied Biosystems Assay ID	Gene name	Gene symbol
Hs00231763_m1	-Homeobox transcription factor or -NK2 transcription factor related, locus 5	Nkx2.5 or NKX2-5 or NKX2.5
Hs00171403_m1	-zinc finger cardiac transcription factor or -GATA binding protein 4	GATA-4 or, GATA4 (AB)
Hs00231149_m1	-myocyte enhancer factor 2C	MEF2c or MEF2C
Hs00361155_m1	-T-box transcription factor or -T-box 5	Tbx5 or TBX5
Hs00542350_m1	-GATA co-factor ("Friend of GATA") or -zinc finger protein, multitype 1	FOG 1 of FOG-1 or FOG1
Hs00251489_m1	-Helix-loop-helix transcription factor -mesoderm posterior 1 homolog (mouse) (AB)	Mesp1 or MESP1
Hs00232018_m1	-GATA binding protein 6 (AB)	GATA-6 or GATA6
Hs00911699_m1	-Kinase insert domain receptor (a type III receptor tyrosine kinase)	Flk-1, or FLK1 or KDR

Table 1

[0115] Left ventricular function and structure were serially followed by transthoracic echocardiography (Sequoia 512; Siemens, Malvern, PA and VisualSonics Inc, Toronto, Canada). Ejection fraction (%) was calculated as [(LVVd - LVVs)/LVVd] × 100, where LVVd is left ventricular end-diastolic volume (μl), and LVVs is left ventricular end-systolic volume (μl).

[0116] Fig. 4 shows the mRNA expression, in arbitrary units (A.U.) of cardiac transcription factors for respectively Nkx2.5 mRNA, GATA-6 mRNA and Fog-1 mRNA, of untreated, naïve hMSC (left) and on CP-hMSC, treated with a cardiogenic cocktail (right). It is clear, in each case, that the results are far better when using cells treated with a cardiogenic cocktail.

[0117] Fig. 5 shows images obtained on confocal microscopy showing nuclear translocation Nkx2.5, MEF2c, GATA4 and FOG-2 polypeptides in naïve CP-hMSC treated with a cardiogenic cocktail. Nkx2.5, MEF2c, GATA4 and FOG-2 appear in green and DAPI in blue. On the images of naïve hMSCs, no transcription factor appears. The polypeptides are translocated on the nuclei of CP-hMSCs (right) as indicated by the concentrated green colour.

[0118] Fig. 6 illustrates the progressive conversion (at days D0, D5, D15, and day D20) from naïve hMSCs to 'cocktail-guided' CP-hMSCs and eventually cardiomyocytes, CM. On D0, nuclei are coloured in blue by DAPI. On D5, MEF2C polypeptide is translocated on nuclei (green). On D15, sarcomeric α-actinin is present (red), which shows that sarcomeres are present and hence that the cells are definitively engaged into the cardiomyocytic differentiation and are no longer cardiopoietic. A large quantity of troponin-1 is present in cardiomyocytes on D20 (terminal differentiation).

[0119] Fig. 7 shows the transition electron microscopy ultrastructure of naïve hMSC (left) and cocktail-guided cardiomyocyte (right). To this end, cells were cultured in 1% platelet lysate for 15 days The cardiomyocytes present a mitochondrial maturation, a sarcomerogenesis and formation of myotubes.

[0120] Fig. 8 shows a cocktail-guided cardiomyocyte in light microscopy. Maturation of the excitation-contraction system was assessed through induction of calcium transients. To this end, cells were cultured for 15 days following 5 days of cocktail stimulation and loaded for 30 min at 37°C with 5 μM of the calcium-selective probe fluo-4-acetoxymethyl ester (Molecular Probes, Carlsbad, CA) for live imaging using a temperature controlled Zeiss LSM 510 microscope (Zeiss) and line-scan images acquired during 1 Hz stimulation.

[0121] Fig. 9 shows a 3-, 8-, and 8-fold increase in Tbx-5, MEF2C and MESP-1 in treated versus untreated hMSC.

[0122] As shown in Fig. 10, CP-hMSCs meeting CARPI criteria, were delivered *in vivo* one-month following infarction and significantly improved ejection fraction over naïve patient-matched hMSC.

[0123] Fig. 11 represents an echocardiography of infarcted hearts untreated (left) and treated with cardiopoietic cells (right), which shows a far better anterior wall reanimation upon treatment with CP-hMSCs. Electrocardiograms were recorded using four-limb-lead electrocardiography (MP150; Biopac, Goleta, CA) under light anaesthesia (1.5% isoflurane).

[0124] On echocardiography, contractility improved by 15% and 20% at one- and two-months, respectively, following treatment with CP-hMSC (n=14), in contrast to marginal change recorded with naïve hMSC (n=17) or sham (n=10; Figure 9). On top: Echocardiography of infarcted hearts 4 weeks following coronary ligation and 1day prior to cellular transplant (4 wks post MI – no Tx) revealed on M-Mode an akinetic anterior wall in both study groups. Middle: 4 weeks after cellular transplantation (4 wks post Cell Tx), naïve hMSC treated hearts demonstrated maintained akinesis in the anterior wall, in contrast to re-animation in the CP-hMSCs treated group. Lower: 8 weeks following cellular transplantation (8 wks post Cell Tx), naïve hMSC treated hearts continued to show limited evidence for myocardial repair *versus* robust contractile activity in the CP hMSC treated infarcted hearts. Left panels represent para-sternal (PS) long axis views, with dash line indicating level of 2-D M-Mode capture. Arrowheads in right panels indicate anterior wall re-animation.

[0125] Fig. 12 shows that on average, guided cardiopoietic hMSC achieved a marked improvement at the one and two month follow-up following injection into infarcted myocardium. In contrast, naïve hMSC or sham controls had limited impact on ejection fraction. Star and double star indicate a $p < 0.01$ over naïve hMSC for the two time points.

[0126] In hearts treated with cardiopoietic cells derived from hMSC, functional improvement correlated on three-month and 18-month histopathological evaluation with myocardial regeneration. Aneurysms and scar, which remained uncorrected in naïve hMSC-treated hearts, were resolved with cardiopoietic hMSC treatment that induced re-muscularization (Fig. 13).

[0127] Gross pathological evaluation demonstrated resolution of scar downstream of left anterior descending (LAD) artery ligation (yellow circle on the hearts) with, on cross-section, robust re-muscularization and diminished remodeling in cardiopoietic (CP, right) in contrast to naïve (left) hMSC-treated infarcted hearts at 6-months following treatment initiation. These results are particularly good.

[0128] Probing for ALU-DNA was performed using human ALU-Probe (Biogenex, San Ramon, CA) by hybridization at 85°C for 5-10 minutes and incubation at 37°C overnight followed by anti-Fluorescein GFP-labeled secondary detection.

[0129] Confocal resolution revealed, in the CP-hMSC-treated murine myocardium, widespread presence of human-derived cells with positive staining for ALU DNA sequences specific to the human species validated with human-specific lamin immunostaining, all absent in infarcted controls (Fig. 14).

[0130] In contrast to sham (left), cardiopoietic hMSC treated hearts on confocal microscopy evaluation revealed dramatic presence of human nuclei as stained by a human h-ALU DNA probe (middle) imbedded within the murine infarcted myocardium, further confirmed with additional staining for a human-specific lamin antibody (right, inset shown in Fig. 14). Frozen myocardial sections were made from super-oxygenated 3% paraformaldehyde in PBS perfusion fixed hearts. Bar indicates 50 μm.

[0131] Fig. 15 shows that human-specific troponin-I antibody revealed no staining in naïve (left) *versus* significant staining in the anterior wall of cardiopoietic hMSC treated hearts (middle and right panels)

[0132] Moreover, as shown in Fig. 16 human troponin-I staining of naïve (top) *versus* cardiopoietic (bottom) hMSC treated hearts, counterstained with mlC2v, demonstrated generation of ventricular myocardium from engrafted human cells. Bars indicate 20 μm (top) and 50 μm (bottom).

[0133] As illustrated in Fig. 17 within the remnant scar of cardiopoietic derived from hMSC-treated hearts, human stem cell derived myocardium could be distinguished from native murine myocardium with human troponin colocalization with mlC2V. Bar indicates 50 μm.

[0134] In Fig. 18 surface microscopy detected angiogenesis distal to the ligated LAD (black circle) in CP-hMSCs-treated hearts arising from the right coronary artery (RCA; left bottom) and circumflex (right bottom).

[0135] Fig. 19 shows confocal evaluation of collateral vessels from cardiopoietic hMSC treated hearts demonstrated human-specific CD-31 (PECAM-1) staining. Bar represents 20 μm.

[0136] Fig. 20 shows the evolution of the change of ejection fraction relative to sham in %, during 12 months, for treatment with both naïve and cocktail-guided (CP) hMSC. Relative to sham, treatment with naïve hMSC showed a 5% and 2.5% ejection fraction effect at 6 and 12 months, respectively.

[0137] In contrast, CP-hMSCs-treated infarcted mice demonstrated significant ejection fraction improvement of 25% at 6 and 12 months relative to sham (Fig. 20). Furthermore, the infarcted cohort was stratified to evaluate efficacy in subgroups with documented overt heart failure (ejection fraction <45%) at the time of intervention. Despite equivalent pre-treatment ejection fraction at 35%, only cardiopoietic hMSC treatment improved absolute ejection fraction by 10% at 6 and 12-months, in contrast to a 5% decline in ejection fraction in the naïve hMSC-treated cohort (Fig. 23). As shown in Fig. 22, superior survival benefit in the cardiopoietic hMSC treated group in contrast to naïve treated cohort and sham was determined through application of the Kaplan-Meier function with censoring.

[0138] Efficacy of cardiopoietic (CP) hMSC was demonstrated by echocardiography at 1-year follow-up (see Fig. 25). Long axis imaging of naïve stem cell treated hearts revealed a fibrotic and hypokinetic anterior wall most evident on apical M-Mode evaluation (Patient 11, left panels). In contrast, CP-hMSC-treated hearts revealed a robust contractile profile throughout the anterior wall reflecting a sustained benefit from guided stem cell therapy (Patient 11, right panels).

Example 2

[0139] Similar results have been observed by treating stem cells with a cocktail containing recombinant TGFβ-1(2.5 ng/ml), BMP4 (5 ng/ml), Cardiotrophin (1ng/ml), Cardiogenol C (100 μM) and α-thrombin, (1 U/ml), FGF-2 (10 ng/ml), IGF-1 (50 ng/ml) and Activin-A (5 ng/ml) used in a combinatorial fashion.

Example 3

[0140] Similar results have been observed by treating stem cells with a cocktail containing recombinant TGF-β1 (2.5 ng/ml), BMP-4 (5 ng/ml), Activin-A (5 ng/ml), FGF-2 (10 ng/ml), IL-6 (100 ng/ml), Factor IIa (hα-thrombin, 1 U/ml), IGF-1 (50 ng/ml), and retinoic acid (1 μM) used in a combinatorial fashion.

OTHER EMBODIMENTS

[0141] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims

CLAIMS

1. A composition comprising TGFβ-1, BMP4, α-thrombin, a compound selected from the group consisting of Cardiotrophin and IL-6, and a compound selected from the group consisting of Cardiogenol C and retinoic acid.
2. The composition of claim 1, which comprises TGFβ-1, BMP4, α-thrombin, Cardiotrophin and Cardiogenol C.
3. The composition of claim 1, which comprises at least one compound selected from the group consisting of FGF-2, IGF-1, Activin-A, TNF-α, FGF-4, LIF, VEGF-A and combinations thereof.
4. The composition of any of the preceding claims, which comprises FGF-2, IGF-1 and Activin-A.
5. The composition of any of the preceding claims, which comprises Activin-A, FGF-2, IL-6, IGF-1 and retinoic acid.
6. The composition of claim 1 which lacks at least one compound chosen in the group consisting of TNF-α, FGF-4, LIF, and VEGF-A.
7. The composition of any of claims 1 to 6, wherein when one compound is present in said composition, it is present in an amount of between 1 and 5 ng of said TGFβ-1 per ml, between 1 and 10 ng of said BMP4 per ml, between 0.5 and 5 ng of said Cardiotrophin per ml, between 0.5 and 5 units of said α-thrombin per ml, and between 50 and 500 nM of said Cardiogenol C, between 1 and 10 ng of said FGF-2 per ml, between 10 and 100 ng of said IGF-1 per ml, between 1 and 50 ng of said Activin-A per ml, between 1 and 50 ng of said TNF-α per ml, between 1 and 20 ng of said FGF-4 per ml, between 10 and 100 ng of said IL-6 per ml, between 1 and 10 units of said LIF per ml, between 1 and 50 ng of said VEGF-A per ml, between 0.1 and 1.0 μM of said retinoic acid per ml.
8. The composition of claim 1 containing recombinant TGFβ-1(2.5 ng/ml), BMP4 (5 ng/ml), Cardiotrophin (1ng/ml), Cardiogenol C (100 μM), used in a combinatorial fashion.
9. The composition of claims 8, further comprising α-thrombin, (1 U/ml), FGF-2 (10 ng/ml), IGF-1 (50 ng/ml) and Activin-A (5 ng/ml).
10. The composition of claim 1containing recombinant TGFβ-1 (2.5 ng/ml), BMP4 (5 ng/ml), Activin-A (5 ng/ml), FGF-2 (10 ng/ml), IL-6 (100 ng/ml), Factor IIa (hα-thrombin, 1 U/ml), IGF-1 (50 ng/ml), and retinoic acid (1 μM) used in a combinatorial fashion.

11. The composition of any of claims 1 to 10 which is comprised in a medium selected from the group consisting of media containing of fetal calf serum, human serum, platelet lysate, and mixtures thereof

12. A method for obtaining, from initial cells, differentiated cells expressing an elevated level of at least one of the mRNAs selected from the group consisting of MEF2c mRNA, MESP-1 mRNA, Tbx-5 mRNA, GATA4 mRNA , Flk-1 mRNA, GATA6 mRNA, Fog-1 mRNA, and combinations thereof, and/or have at least one polypeptide selected from the group consisting of Nkx2.5 polypeptides, MEF2C polypeptides, Tbx-5 polypeptides, FOG-2 polypeptides, GATA-4 polypeptides MESP-1 polypeptides, and combinations thereof, wherein said at least one polypeptide is associated with the nuclei of said differentiated cells, wherein said method comprises culturing initial cells in the presence of a composition according to any of claims 1 to 11.

13. Method of claim 12 wherein the differentiated cells express an elevated level of MEF2c mRNA and MESP-1 mRNA.

14. Method of any of claims 12 and 13, wherein the initial cells are mesenchymal stem cells.

15. Method of claim 14 wherein the mesenchymal stem cells are bone marrow-derived stem cells.

16. Method of any of claims 14 and 15, wherein said mesenchymal stem cells express CD90, CD105, CD133, CD166, CD29, and CD44 on the cell surface and do not express CD14, CD34, and CD45 on the cell surface.

17. Method of any of claims 12 to 16, wherein the differentiated cells are cardiopoietic cells.

18. Cells obtained by the method of any of claims 12 to 17.

19. Cells according to claim 18 wherein these cells are cardiopoietic.

20. Method for delivering differentiated cells to a mammal, wherein said method comprises:

(a) determining that a sample of cells from a population of differentiated cells comprises cells that express an elevated level of at least one of the mRNA selected from the group consisting of MEF2c mRNA, MESP-1 mRNA, Tbx-5 mRNA, GATA4 mRNA , Flk-1 mRNA, GATA6 mRNA, Fog-1 mRNA, and combinations thereof, and/or have at least one polypeptide selected from the group consisting of Nkx2.5 polypeptides, MEF2C polypeptides, Tbx-5 polypeptides, MESP-1 polypeptides, GATA-4 polypeptides, FOG-2 polypeptides,

and combinations thereof, wherein said polypeptide is associated with the nuclei of said differentiated cells, and

(b) administering cells from said population of differentiated cells to said mammal.

21. Method of claim 20 wherein said population of differentiated cells was obtained from original cells of any of claims12 to 16 cultured in the presence of a composition of any of claims 1 to 11.

22. Method of any of claims 20 and 21, wherein said step (a) comprises using a reverse transcription polymerase chain reaction.

23. Method of any of claims 20 to 22, wherein said administering step comprises administering said cells via an administration selected from the group consisting of systemic, intracardiac, and intracoronary administrations.

24. Method of any of claims 20 to 23, wherein said step (a) comprises using immunocytochemistry.

25. Method for providing heart tissue with cardiomyocytes, wherein said method comprises administering, to said heart tissue, differentiated cells of claims 12 to 16 obtainable by contact with a composition according to claims 1 to 11.