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(54) **Title**: METHODS OF IMPROVING TRANSPLANTATION USING SDF-1ALPHA

(57) **Abstract**: A method of enhancing transplantation of a cell, a tissue or an organ graft in a subject in need thereof is disclosed. The method comprising administering to a subject who has undergone transplantation of a cell, a tissue or an organ graft a therapeutically effective amount of SDF-1alpha, thereby enhancing transplantation of the cell, the tissue or the organ graft in the subject.

METHODS OF IMPROVING TRANSPLANTATION USING SDF-1ALPHA

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to the use of SDF-1α for improving transplantation of cells, tissues or organ grafts including fat grafts.

New blood vessel formation (neovascularization) in transplanted organs prevents tissue necrosis and rejection. Postnatal neovascularization entails mobilization of endothelial progenitor cells (EPCs) from the bone marrow (BM). Basic studies suggest that local or systemic administration of cultured or fresh EPCs enhances ischemic neovascularization and improves function of ischemic tissues in animals with hindlimb or myocardial ischemia. In addition, clinical studies related to therapeutic angiogenesis using autologous EPCs or BM mononuclear cells (BM-MNCs) demonstrated the beneficial effect and safety of this technique in patients with severe ischemia in the lower limb [Tateishi-Yuyama E et al., Lancet (2002) 360 (9331):427-35] and with acute myocardial infarction [Assmus B et al., Circulation (2002) 106(24):3009-17].

Patients with diabetes mellitus often have concurrent severe peripheral vascular disease and reduced collateral formation, which can lead to vital organ damage. Recent data indicate that such patients also had reduced numbers of EPCs and that these EPCs were impaired [Tepper et al. Circulation (2002) 106:2781-6]. Consequently, diabetes patients could be at high risk of delayed wound healing and organ transplant rejection. The standard treatment for neovascularization to prevent organ transplant rejection using autologous EPC therapy might therefore be hampered in diabetic patients. Methods for increasing EPC recruitment from the BM, and for boosting their number and functionality in the peripheral circulation are currently being sought.

Because it bypasses complications of transplanted tissue rejection, autologous fat transplantation is an optimal and commonly used technique for tissue augmentation, smoothing of deep wrinkles, and for restoring soft tissue defects due to trauma. However, the results of long-term follow-up studies indicate that, because the grafted fat tissue is poorly vascularized in the early period (2-3 weeks) after its transplantation, up to 80 % of the volume of the transplanted fat is resorbed [Nguyen A et al., Plast Reconstr Surg. (1990) 85(3):378-86]. Since approximately 10 % of the population worldwide is diabetic, there is almost no data on the success rate of autologous fat transplantation in

diabetic patients, however, it is assumed that about 10 % of individuals who undergo autologous fat transplantation have diabetes mellitus, and that in those patients whose neovascularization is disrupted, fat resorption may be exacerbated. Several potential approaches, using healthy animal models, have been proposed to overcome the reduced vascularization of grafted fat after its transplantation, and these include either its co-transplantation with EPCs [Yi et al. Dermatol Surg. (2006) 32(12):1437-43] or local delivery of vascular endothelial cell growth factor (VEGF) using gene therapy methods [Lu F et al., Plast Reconstr Surg (2009) 124:1437-1446], resulting in EPC mobilization from the BM into the peripheral blood (PB) and finally to the transplanted fat tissues.

The stromal cell-derived factor-1α (SDF-1α, GenBank Accession Nos. NM_000609 and NM_199168), also referred to as CXCL12, is produced by many cell types, including bone marrow stromal cells, astrocytes and endothelial cells. SDF-1α typically binds CXCR4, also known as fusin or LESTR, which has a wide cellular distribution, with expression on most immature and mature hematopoietic cell types, vascular endothelial cells and neuronal/nerve cells. During embryogenesis, the SDF-1α/CXCR4 pathway is critical for hematopoiesis, and for vascular and cardiac development. Increasing evidence has demonstrated that hematopoietic stem cells and EPCs express CXCR4, and that VEGF stimulates CXCR4 in endothelial cells. Moreover, SDF-1α is essential for EPC homing, mobilization, and differentiation [Lapidot et al. Blood. (2005) 106(6):1901-10]. It induces the expression of VEGF, and stimulates angiogenesis in vivo [Salcedo R et al., Am J Pathol. (1999) 154(4):1125-35]. Recent reports demonstrated that local delivery of the SDF-1α protein [Yamaguchi J. et al. Circulation. (2003) 107(9):1322-8] or local SDF-1α gene transfer [Hiasa K. et al., Circulation. (2004) 109(20):2454-61] enhanced neovascularization of ischemic hindlimb animal models through an EPC-mediated mechanism.

U.S. Patent Application No. 2010/0166717 discloses methods of treating ischemic disorders and tissue injuries by administering SDF-1 to ischemic tissues of the subject in an amount effective for inhibiting cell apoptosis.

PCT Publication No. 2008/019434 discloses use of agents to enhance adipogenesis and to promote fat graft survival. According to their teachings, growth factors [e.g. platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and/or fibroblast growth factor (FGF)] are delivered by local or sustained

administration to enhance angiogenesis in association with adipogenesis and to promote fat graft survival.

Additional background art includes U.S. Patent Application No. 20050136042.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a method of enhancing transplantation of a cell, a tissue or an organ graft in a subject in need thereof, the method comprising administering to a subject who has undergone transplantation of a cell, a tissue or an organ graft a therapeutically effective amount of SDF-1alpha, thereby enhancing transplantation of the cell, the tissue or the organ graft in the subject.

According to an aspect of some embodiments of the present invention there is provided a method of enhancing survival of a cell, a tissue or an organ graft in a subject in need thereof, the method comprising: (a) implanting a cell, a tissue or an organ graft into the subject; and (b) administering to the subject a therapeutically effective amount of SDF-1alpha, thereby enhancing survival of the cell, the tissue or the organ graft in the subject.

According to an aspect of some embodiments of the present invention there is provided a method of enhancing survival of a cell, a tissue or an organ graft in a subject in need thereof, the method comprising: (a) contacting a cell, a tissue or an organ graft with SDF-1alpha; and (b) implanting the cell, the tissue or the organ graft into the subject, thereby enhancing survival the cell, the tissue or the organ graft in the subject.

According to an aspect of some embodiments of the present invention there is provided a use of SDF-1alpha for enhancing survival of a cell, a tissue or an organ graft in a subject in need thereof.

According to an aspect of some embodiments of the present invention there is provided a pharmaceutical composition comprising a cell, a tissue or an organ graft, SDF-1alpha and a pharmaceutically acceptable carrier or diluent.

According to an aspect of some embodiments of the present invention there is provided a method of enhancing survival of a fat cell or fat tissue in a subject in need thereof, the method comprising: (a) implanting a fat cell or fat tissue into the subject;

and (b) administering to the subject a therapeutically effective amount of SDF-1alpha, thereby enhancing survival of the fat cell or fat tissue.

According to some embodiments of the invention, the cell or the tissue is a fat cell or fat tissue.

According to some embodiments of the invention, the cell or the tissue is a beta cell graft.

According to some embodiments of the invention, the subject has diabetes.

According to some embodiments of the invention, the fat cell or fat tissue is for treating a soft tissue defect.

According to some embodiments of the invention, the soft tissue defect is selected from the group consisting of a skin condition, a skin malady, a wound, a burn, a cancer, a surgery, a reconstruction surgery, a skin depression, a congenital malformation and an acquired disease.

According to some embodiments of the invention, the method further comprises contacting the cell, the tissue or the organ graft with SDF-1alpha prior to the implanting.

According to some embodiments of the invention, the subject is treated with a therapeutically effective amount of SDF-1alpha prior to the implanting of the cell, the tissue or the organ graft.

According to some embodiments of the invention, the method further comprises administering a therapeutically effective amount of SDF-1alpha to the subject following the implanting.

According to some embodiments of the invention, the administering is effected by direct injection of the SDF-1alpha into the cell, the tissue or the organ graft.

According to some embodiments of the invention, the dose of the SDF-1alpha is about 1 pg/1,000,000 cells to about 1 mg/1,000,000 cells per injection.

According to some embodiments of the invention, the administering the SDF-1alpha is effected by a systemic route.

According to some embodiments of the invention, the dose of the SDF-1alpha is about 1 pg/kg to about 1 mg/kg body weight.

According to some embodiments of the invention, the therapeutically effective amount is for promoting angiogenesis and reducing apoptosis of the cell, the tissue or the organ graft.

According to some embodiments of the invention, the subject is diabetic.

According to some embodiments of the invention, the subject is a human subject.

According to some embodiments of the invention, the cell, the tissue or the organ graft is selected from the group consisting of a cardiac, a retina, a liver, a kidney, a lung, a splenic, a bone, a cartilage and a lymph cell, tissue or organ.

According to some embodiments of the invention, the tissue or the organ graft is a solid tissue or a solid organ.

According to some embodiments of the invention, the cell, the tissue or the organ graft is from an autologous origin.

According to some embodiments of the invention, the method further comprises administering to the subject endothelial precursor cells (EPCs).

According to some embodiments of the invention, the method further comprises administering to the subject at least one factor selected from the group consisting of an extracellular matrix component, a growth factor, a hormone, an angiogenic factor, a coagulation factor, a cytokine, a chemokine, an enzyme, a neurotransmitter, a vitamin, a carbohydrate, an ion, an iron chelator, a fatty acid, an antibiotic and an amino acid.

According to some embodiments of the invention, the pharmaceutical composition further comprises at least one factor selected from the group consisting of an extracellular matrix component, a growth factor, a hormone, an angiogenic factor, a coagulation factor, a cytokine, a chemokine, an enzyme, a neurotransmitter, a vitamin, a carbohydrate, an ion, an iron chelator, a fatty acid, an antibiotic, and an amino acid.

According to some embodiments of the invention, the dose of the SDF-1alpha in the pharmaceutical composition is about 1 pg/kg to about 1 mg/kg body weight.

According to some embodiments of the invention, the method further comprises contacting fat cell or fat tissue with SDF-1alpha prior to the implanting.

According to some embodiments of the invention, the administering is effected systemically.

According to some embodiments of the invention, the administering is effected locally.

According to some embodiments of the invention, the fat cell or fat tissue is for treating a soft tissue defect.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs. 1A-T are photographs depicting mice 15 weeks after fat transplantation. Figure 1A is a photograph of a representative mouse at day 1 post fat transplantation; Figures 1B-D are photographs of a representative mouse undergoing a fat graft dissection 15 weeks post fat transplantation; Figures 1E-L are photographs of representative non-homogenous lumps in the scalps of mice from the different treatment groups at week 15 (as indicated in the photographs); and Figures 1M-T are photographs of representative fat grafts after dissection from the different treatment groups (as indicated in the photographs).

FIGs. 2A-D are graphs depicting fat weight and volume. Figure 2A-B are graphs of mean weights of fat grafts from non-diabetic mice (Figure 2A) and from diabetic mice (Figure 2B); and Figure 2C-D are graphs of mean volumes of fat grafts from non-diabetic mice (Figure 2C), and from diabetic mice (Figure 2D). *$P<0.05$, **$P<0.001$, †$P<0.001$.

FIGs. 3A-D are graphs depicting EPC number in bone marrow mononuclear cells (BM-MNCs) and in peripheral blood mononuclear cells (PB-MNCs). Figures 3A-B are graphs of mean EPC number from BM (Figure 3A) and from PB (Figure 3B) calculated

by double staining with acLDL and BS-1 lectin; Figures 3C-D are graphs of mean EPC number from BM (Figure 3C) and from PB (Figure 3D) calculated by FACS using Sca-1 and Flk-1 double staining. *P<0.05, **P<0.001, †P<0.001.

FIGs. 4A-Q are photographs depicting morphology and characterization of EPCs. Endothelial progenitor cells (EPCs) were isolated from PB-MNCs of non-diabetic mice and cultured for four days. Figures 4A-C are photographs of uptake of acetylated LDL by spindle-shaped adherent EPCs, FITC-conjugated lectin BS-1 binding on the EPC cell surface, and the merged image, respectively; Figures 4D-F are photographs of immunofluorescence detection of CD31 (red, Figure 4D) and CD34 (green, Figure 4E) on the EPC surface, and the merged image (Figure 4F); Figures 4G-I are photographs of immunofluorescence detection of Sca-1 (red, Figure 4G), Flk-1 (green, Figure 4H) and merged image (Figure 4I). The EPC nuclei are blue because the cells were counterstained with the fluorescent DNA dye DRAQ5™. Scale bar: 20 μm; Figures 4J-Q are photographs of double staining with acLDL and lectin BS-1 binding on the EPC cell surface, isolated from PB-MNCs of mice treated as indicated.

FIGs. 5A-B are graphs depicting the effect of SDF1α on MVD in fat grafts following fat transplantation. Each bar represents the mean MVD ± SD from five fields of the pooled data in fat grafts of non-diabetic mice (Figure 5A) and diabetic mice (Figure 5B). *P<0.05, **P<0.001, †P<0.001.

FIGs. 6A-H are photographs depicting histological sections of fat grafts 15 week post fat transplantation. Sections were stained with hematoxylin and eosin and then examined under a light microscope for (a) integration, as evidenced by the presence of intact and nucleated fat cells in an organized architecture, (b) fibrosis, as evidenced by the presence of collagen and elastic fibrils, (c) the presence of cysts and vacuoles, and (d) inflammation, as evidenced by the extent of lymphocyte and macrophage infiltration. Each criterion was graded on a scale of 0 to 5 where 0 = absence, 1 = minimal presence, 2 = minimal to moderate presence, 3 = moderate presence, 4 = moderate to extensive, and 5 = extensive presence. Figures 6A-H are representative micrographs as follows: from a PBS-treated fat graft in a non-diabetic mouse (Figure 6A), from a SDF1α-treated fat graft in a non-diabetic mouse (Figure 6B), from an EPC-treated fat graft in a diabetic mouse (Figure 6C) and from a SDF1α-treated fat graft in a diabetic mouse (Figure 6D); Figures 6E-H are representative micrograph of CD31-stained sections as follows: a PBS-

treated fat graft of a non-diabetic mouse (Figure 6E), a SDF1α-treated fat graft in a non-diabetic mouse (Figure 6F), an EPC-treated fat graft in a diabetic mouse (Figure 6G) and a SDF1α-treated fat graft in a diabetic mouse (Figure 6H). Scale bar: 200 μm.

FIGs. 7A-B are graphs depicting the effect of SDF1α on angiogenic and apoptotic factors in the fat grafts. Figure 7A is a graph depicting western blot analysis of protein expression of the angiogenic factors VEGF, CXCR4, AKT and eNOS in the fat grafts in diabetic mice; and Figure 7B is a graph depicting western blot analysis of protein expression of the apoptotic factors caspase 3 and cytochrome c in the fat grafts in diabetic mice. Each bar represents the mean protein expression of each factor ± SD in the fat grafts. *P<0.05, **P<0.001, †P<0.001, •P<0.001.

FIGs. 8A-B are graphs depicting the effect of SDF1α on in-vivo EPC migration and in-vitro EPC tube formation in matrigel. Figure 8A, the feeder trays were coated with 100 μl of EBM-2 containing 200 ng/ml of SDF1α. EPC migration was allowed for 12 hours after plating them on a Boyden membrane chamber. Extent of migration was assessed by the intensity of the fluorescence signal as measured by a fluorescence spectrophotometer, and the results were expressed as the average of relative fluorescence units (RFU). Each bar represents the mean RFU percentage of non-diabetic control ± SD in the Boyden chamber for each group. Figure 8B depicts EPC tube formation on matrigel *in vitro* with the different treatments. Each bar represents the mean percentage of tube formation score of non-treated control EPCs ± SD for each treatment. **P<0.001

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to the use of SDF-1α for improving transplantation of cells, tissues or organ grafts including fat grafts.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Fat transplant procedures are often compromised by high resorption rates of the autologous transplanted fat, thus, the reduction of fat resorption to improve the procedure outcome has become a clinical challenge. Adequate neovascularization of the transplanted fat is essential for the long-term retention of the fat graft. Stromal derived factor-1 alpha (SDF-1α) is a chemokine which induces neovascularization by mobilizing endothelial progenitor cells (EPCs).

While reducing the present invention to practice, the present inventors have uncovered that SDF-1α improves long-term fat graft retention, particularly in diabetes patients where neovascularization is specifically impaired.

As is illustrated hereinbelow and in the Examples section which follows, the present inventors have utilized a nude mouse model (diabetic and non-diabetic mice) and injected subcutaneously thereto human fat tissue. The fat grafts were treated with either EPCs (in diabetic mice) or with SDF-1α (in diabetic and non-diabetic mice) and the results were compared to PBS-treated fat grafts of non-diabetic and diabetic mice. As is evident from the results, the present inventors have shown that in diabetic mice the transplanted fat was fully resorbed (Figures 1A-T), however, the weight and volume of the SDF-1α -treated grafts were higher than those of EPC- and PBS-treated fat grafts in both diabetic and non-diabetic mice (Figures 1A-T and Figures 2A-D). SDF-1α treatment significantly increased the frequency of circulating endothelial progenitor cells (Figures 3A-D) and microvascular density (MVD, Figures 5A-B and Figure 7A) while reducing apoptosis (Figure 7B). Furthermore, SDF-1α treatment led to higher integration and less inflammation of the fat grafts (Figures 6A-H and Table 2). Taken together, these results substantiate the value of SDF-1α treatment in enhancing long-term retention of grafts such as fat transplants.

Thus, according to one aspect of the present invention there is provided a method of enhancing survival of a cell, a tissue or an organ graft in a subject in need thereof, the method comprising: (a) implanting a cell, a tissue or an organ graft into the subject; and prior to, concomitantly with, or following (b) administering to the subject a therapeutically effective amount of SDF-1α.

According to a specific embodiment, SDF-1α is administrated following transplantation of a cell, tissue or organ graft.

The term "a cell, a tissue or an organ graft" as used herein refers to a cell, group of cells, bodily tissue or organ which may be transplanted in full or in part, including solid tissues and soft tissues. Exemplary cells, tissues or organs which may be transplanted according to the present teachings include, but are not limited to, liver, pancreas, spleen, kidney, heart, lung, skin, intestine, retina, bone, cartilage, fat and lymphoid/hematopoietic (e.g. lymph node, Peyer's patches thymus or bone marrow) cells, tissues or organs..

According to one embodiment, the cell or tissue is a fat cell or fat tissue.

Thus, according to a specific embodiment, there is provided a method of enhancing survival of a fat cell or fat tissue in a subject in need thereof, the method comprising: (a) implanting a fat cell or fat tissue into the subject; and (b) administering to the subject a therapeutically effective amount of SDF-1alpha.

The terms "fat cell" or "fat tissue" as used herein refer to any cell or group of cells composed in a fat tissue, including for example, lipocytes, adipocytes, adipocyte precursors including pre-adipocytes and mesenchymal stem cells. It will be appreciated that according to the present teachings, the fat cells may be dispersed or may be comprised in a tissue.

According to one embodiment, the cell or tissue is a beta cell graft.

As used herein, the term "beta cell graft" refers to the pancreatic islet endocrine cells capable of producing and secreting insulin (e.g. in response to physiological signals such as elevated glucose concentrations) and expressing typical beta cell markers including, but not limited to, insulin, pdx, Hnf3β, PC1/3, Beta2, Nkx2.2, GLUT2 and PC2.

The cell, tissue or organ graft may be naïve i.e., not subject to treatment or culturing prior to implantation, or a treated cell, tissue or organ graft which has been subject to culturing or genetic modification.

The graft can be of fully differentiated cells (i.e., fetal or adult somatic tissue) or can be partially or terminally differentiated.

According to one embodiment, the tissue or organ comprises a solid tissue or organ.

According to another embodiment, the cells comprise isolated cells. The isolated cells may be of homogeneous or heterogeneous nature.

The number of cells may vary over a wide range and one of ordinary skill in the art will recognize that this number will vary depending upon the type and size of the area to be treated, the relative degree of vascularization of the area to be treated, the age of the subject to be treated and the relative viability of the cells available for transplantation. It will be appreciated that the number of cells transplanted may be adjusted according to the procedure used, the site of injection and the relative vascularization of the site to be injected. One of ordinary skill in the art will recognize that certain conditions may necessitate the adjustment of the cell numbers outside of the below described ranges.

According to some embodiments of the present invention, the number of fat cells for transplantation range from about 100 to about 1000 cells per 1 ml, 100 to about 10,000 cells per 1 ml, 100 to about 100,000 cells per 1 ml, 100 to about 1,000,000 cells per 1 ml, about 1,000 to about 100,000 cells per 1 ml, about 1,000 to about 1,000,000 cells per 1 ml, about 10,000 to about 1,000,000 cells per 1 ml, about 10,000 to about 10,000,000 cells per 1 ml, about 10,000 to about 100,000,000 cells per 1 ml, about 100,000 to about 10,000,000 cells per 1 ml, or about 1,000,000 to about 10,000,000 cells per 1 ml.

According to another embodiment about 0.01 to 1 ml, about 0.01 to 2 ml, about 0.01 to 3 ml, about 0.01 to 10 ml, about 0.01 to 100 ml, about 0.01 to 200 ml, about 0.01 to 1000 ml, about 0.01 to 2000 ml, about 0.01 to 3000 ml, about 1 to 100 ml, about 1 to 200 ml, about 1 to 1000 ml, about 1 to 2000 ml, about 1 to 3000 ml, about 10 to 100 ml, about 10 to 200 ml, about 10 to 1000 ml, about 10 to 2000 ml, about 10 to 3000 ml, about 100 to 200 ml, about 100 to 1000 ml, about 100 to 2000 ml or about 100 to 3000 ml of fat tissue are transplanted.

It will be appreciated that the subject may be administered a single transplantation or several transplantations along a period of time (e.g. about 2, 5, 10, 20, 50, 100 or more transplantation procedures), as described in further detail hereinbelow.

The phrase "survival" as used herein refers to the ability of the cell, tissue or organ graft to remain viable and intact following engraftment thereof. Preferably, the cell, tissue or organ graft survives for a period of a few days, a few weeks, a few months or a few years following engraftment thereof.

As used herein, the term "enhancing" in respect to survival of the cell, tissue or organ graft refers to a process of increasing the life span of cells in the graft and/or decreasing the number of cells which undergo resorption, apoptosis or cell death within the graft. Thus in some embodiments of the present invention, enhancing refers to at least about 10 %, 20 %, 50 %, 80 %, 90 % increase in viable cells and/or at least about 10 %, 20 %, 50 %, 80 %, 90 % arrest in cell death. Those of skill in the art will understand that various *in vitro* and *in vivo* methodologies and assays can be used to assess cell viability, and similarly, various methodologies and assays may be used to assess cell death or cell apoptosis. Thus, for example, assessment may be carried out in a cell or tissue sample of the transplanted subject *in vitro* by FACS analysis, terminal deoxyuridine triphosphate nick end labeling (TUNEL) assay and/or cell viability assays e.g. MultiTox Assays. Alternatively, assessment of graft survival may be carried out *in vivo* by invasive or non-invasive mechanisms, as for example, using magnetic resonance (MR) for detection of labeled cells (e.g. iron-labeled cells) transplanted into a subject (e.g. using a clinical 3.0-T magnetic resonance scanner).

Enhancing survival of the cell, tissue or organ graft according to the present teachings is achieved by administering to the subject SDF-1α.

As used herein the term "SDF-1α" (stromal cell-derived factor-1 alpha) refers to at least an active portion of a mammalian (e.g., human) C-X-C chemokine polypeptide (also designated CXCL12) having at least one functional property of SDF-1α (e.g., chemotaxis or binding to CXCR4). Preferably the SDF-1α of the present invention is capable of increasing the frequency of circulating endothelial progenitor cells (EPCs) in the subject being transplanted and increasing the microvascular density (MVD) and reducing apoptosis of the graft as further described hereinbelow. Preferably, the SDF-1α of the present invention is also capable of increasing integration and decreasing inflammation of the graft (see Example 1 of the Examples section which follows). Exemplary SDF-1α of the present invention are set forth in GenBank Accession Nos. NP_954637.1, NP_000600.1, NP_001029058.1, NP_001171605.1.

Any SDF-1α known in the art can be used in accordance with the teachings of the present invention. Furthermore, the SDF-1α used in accordance with the present invention may be recombinant SDF-1α or purified SDF-1α. For instance, exemplary SDF-1α which may be used in accordance with the present invention include, but are

not limited to, recombinant human SDF-1α (CXCL12) available from Sigma Aldrich (MO, USA), from ProSpec-Tany TechnoGene Ltd, or from Cell Sciences.

As used herein, the phrase "subject in need thereof" refers to a mammal, preferably a human being, male or female at any age that is in need of a cell, tissue or organ transplantation. Typically the subject is in need of a cell, tissue or organ transplantation (also referred to herein as recipient) due to a disorder or a pathological or undesired condition, state, or syndrome, or a physical, morphological or physiological abnormality which is amenable to treatment via a cell, tissue or organ transplantation. Examples of such disorders or conditions are provided below.

According to one embodiment of the present invention, the subject has diabetes.

As used herein "diabetes" refers to a disease resulting either from an absolute deficiency of insulin (type 1 diabetes) due to a defect in the biosynthesis or production of insulin, or a relative deficiency of insulin in the presence of insulin resistance (type 2 diabetes), i.e., impaired insulin action, in an organism. The diabetic patient thus has absolute or relative insulin deficiency, and displays, among other symptoms and signs, elevated blood glucose concentration, presence of glucose in the urine and excessive discharge of urine. Diabetes is typically associated with massive loss of pancreatic beta cells. Furthermore, diabetic subjects often have diabetic eye disease (e.g. diabetic retinopathy, cataract or glaucoma) as well as peripheral vascular disease and reduced collateral formation leading to conditions such as delayed wound healing and organ transplant rejection.

Thus, diabetes subject may require transplantation of beta cells (e.g. to replace those destroyed by the disease), of retinal cells (e.g. for treatment of diabetic eye disease as detailed above), of blood vessels (e.g. in peripheral arterial disease), of hair (e.g. for restoration of bald or balding areas, eyelashes, eyebrows, beard hair, chest hair or pubic hair or to fill in scars caused by accidents or surgery such as face-lifts and previous hair transplants) and/or of fat cells or tissues (e.g. for treatment of uncured wounds, for filling of lost tissue, etc. as detailed in further detail below). These subjects are especially amenable to treatment by the present methods.

The inventors of the present invention have shown that administration of SDF-1α along with transplantation of a graft (e.g. fat graft) results in high vascularization of the graft and graft integration in diabetes subjects (see Example 1 of the Examples section

which follows). Thus, according to the present methods, any transplant, including a fat cell or tissue graft, a beta cell graft, a retinal cell graft or a blood vessel graft may be administered to a diabetes subject along with SDF-1α essentially as taught herein, in order to enhance engraftment thereof.

According to another embodiment, the subject has a soft tissue defect.

In general, fat transplantation may be used to treat any soft tissue defect, to fill any soft tissue deficit and for augmentation of external and internal surfaces and structures of the body which are missing due to surgery, as a result of aging of a tissue, or due to disease, trauma or an injury. Examples include, but are not limited to, urological surgeries, tumor removal surgeries, reconstructive surgeries and skin surgeries. Likewise, fat transplantation may be used as an alternative to silicone or collagen fillers. Fat transplantation may be used to fill depressions (i.e. areas of the body which are hollow or sunken and lack the cellular substance, body or volume compared to the same area on a normal body) after injury or pursuant to surgical procedures such as cosmetic surgery, including, but not limited to, facelifts, mastectomies or lumpectomies and due to other procedures, as for example, removal of cancerous tissues, especially tumors at or near the skin of the subject. Fat transplantation may also be used in numerous other applications, including urological procedures involving the buildup of weak or damaged structural tissue, in treatment of wrinkles, burns, skin conditions, skin maladies and wounds and to augment areas of the body, such as the buttocks, biceps, triceps muscles, calf muscles, breasts, hands and penis. Furthermore, fat transplantation may be used to treat congenital malformations such as Hemifacial microsomia and acquired diseases such as Romberg's lipodystrophy and Acquired immune deficiency syndrome (AIDS).

According to a specific embodiment, fat cell or tissue transplantation is carried out in a diabetic subject.

Transplanting the cell, tissue or organ may be effected in numerous ways, depending on various parameters, such as, for example, the graft type; the type, stage or severity of the disease or condition; the recipient's organ failure; the physical or physiological parameters specific to the subject; and/or the desired therapeutic outcome. Depending on the application and purpose, transplanting the cell, tissue or organ may be effected using a cell, tissue or organ originating from any of various mammalian species;

by implanting the cell, tissue or organ into various anatomical locations of the subject; using cells, or using a whole or partial organ or tissue; and/or by using a transplant consisting of various numbers of discrete organs, tissues, and/or portions thereof.

Depending on the application, the method may be effected using a cell, tissue or organ which is asyngeneic or non-syngeneic with the subject.

As used herein, a cell, tissue or organ which is "syngeneic" with the subject refers to a cell, tissue or organ which is derived from an individual who is essentially genetically identical with the subject. Typically, essentially fully inbred mammals, mammalian clones, or homozygotic twin mammals are syngeneic.

Examples of syngeneic cells, tissues or organs include cells, tissues or organs derived from the subject (also referred to in the art as "autologous"), a clone of the subject, or a homozygotic twin of the subject.

Thus, for example, in cases where an autologous fat transplant is carried out, the autologous fat cells are typically taken from a subject to fill in depressions or soft tissue deficits in the body of the same subject in an area of the body other than that site from which the fat cells were removed.

As used herein, a cell, tissue or organ which is "non-syngeneic" (or non-autologous) with the subject refers to a cell, tissue or organ which is derived from an individual who is allogeneic or xenogeneic with the subject's lymphocytes.

As used herein, a cell, tissue or organ which is "allogeneic" with the subject refers to a cell, tissue or organ which is derived from a donor who is of the same species as the subject, but which is substantially non-clonal with the subject. Typically, outbred, non-zygotic twin mammals of the same species are allogeneic with each other.

As used herein, a cell, tissue or organ which is "xenogeneic" with the subject refers to a cell, tissue or organ which substantially expresses antigens of a different species relative to the species of a substantial proportion of the lymphocytes of the subject. Typically, outbred mammals of different species are xenogeneic with each other. Typically xenogeneic cells, tissues or organs are obtained from a mammal, such as e.g. of a porcine origin.

Thus, in cases where a non-autologous fat transplant is carried out, the fat cells may be obtained from a subject of the same species as the recipient subject (i.e. allogeneic fat cells as for example from a human donor to a human recipient) or from a

different species (i.e. xenogeneic fat cells as for example from a porcine donor to a human recipient). Such methods are well known to one of ordinary skill in the art.

Regardless of the origin of the cell, tissue or organ graft it is preferably obtained from a source which is known to be substantially pathogen-free.

Depending on the application and available sources, the cell, tissue or organ may be obtained from a prenatal organism, postnatal organism, an adult or a cadaver donor.

A cell, tissue or organ derived from a prenatal organism may be obtained from a fetus at any gestational stage of pregnancy. It will be understood by one skilled in the art that a period of gestation corresponds to a time-period elapsed since fertilization of a developing embryo or fetus. Thus, the stage of differentiation of a developing organ corresponds to the developmental stage of the embryo or fetus from which it is derived. The ordinarily skilled artisan will possess the necessary expertise for suitably obtaining a cell, tissue or organ at a specific gestational stage so as to enable the practicing of the present invention.

It will be appreciated that the cell, tissue or organ according to the present invention may also be obtained from a postnatal organism. Thus, the cell, tissue or organ may be obtained from an organism during the period beginning immediately after birth and extending for about six weeks.

Furthermore, according to the present teachings, the cell, tissue or organ may be obtained from an adult, either a living or cadaver donor. If the cell, tissue or organ is obtained from a cadaver donor, it is best to obtain the organ within 36-50 hours of death as to enable optimal chances of engraftment and functionality. In order to minimize rejection of transplanted cells, tissues or organs, it will be appreciated that factors such as blood type and tissue type should be considered prior to transplantation.

Various common art methods may be employed to obtain a cell, tissue or organ for transplantation.

Thus, for example, fat cells are generally obtained by removing same (e.g. by suctioning) from subcutaneous fat layers in the area of the stomach, legs or other areas where significant fat cells may be found. Preferably the fat cells of the present invention are substantially free of unrelated cells such as erythrocytes, other blood cells, fibroblasts and other cells which may contaminate the fat cells. Furthermore, as the fat

cells are used for transplantation, these cells are kept in a sterile environment until used for transplantation.

It will be appreciated that the fat cells may be further separated from other components which may be found in the aspirated fat, such as, for example, triglycerides, lysozomes, other cellular fragments, blood components, blood cells and large connective tissue fragments, among other less desirable components, before use. Any methods known in the art may be used to separate the fat cells from these other components, but preferably, at least one centrifugation step is employed.

Transplanting a cell, tissue or organ of the present invention may be effected by transplanting the cell, tissue or organ into any one of various anatomical locations, depending on the application. The cell, tissue or organ may be transplanted into a homotopic anatomical location (a normal anatomical location for the transplant), or into an ectopic anatomical location (an abnormal anatomical location for the transplant). Depending on the application, the graft may be advantageously implanted under the renal capsule, or into the kidney, the testicular fat, the sub cutis, the omentum, the portal vein, the liver, the spleen, the heart cavity, the heart, the chest cavity, the lung, the pancreas and/or the intra abdominal space.

Optionally, when transplanting an organ of the present invention into a subject having a defective organ, it may be advantageous to first at least partially remove the failed organ from the subject so as to enable optimal development of the transplant, and structural/functional integration thereof with the anatomy/physiology of the subject.

For example, implanting fat cells according to the present teachings may be carried out by any method known in the art, such as for example, by injection thereof into the desired location, by microsurgery and by surgery in cases were a large amount of fat cells or fat tissue is being transplanted.

According to one embodiment, the fat cells are immediately implanted into a subject. Preferably the fat cells are implanted within 30 minutes, within an hour, within two hours, within three hours, within four hours or within one day of collection (see e.g. Example 1, of the examples section which follows). It will be appreciated that the fat cells of the present invention may be preserved for longer periods of time prior to translation in, for example, by freezing in liquid nitrogen.

Following transplantation of the organ into a subject according to the present teachings, it is advisable, according to standard medical practice, to monitor the growth, functionality and immuno-compatability of the cell, tissue or organ according to any one of various standard art techniques. For example, monitoring the size and integration of the fat graft may be monitored via computerized tomography, by magnetic resonance (e.g. MRI), by ultrasound imaging, by assessment of a physician (e.g. surgeon) or by any other acceptable method.

According to an aspect of the present invention, following implantation of the cell, tissue or organ graft, the subject is administered SDF-1α.

It will be appreciated that SDF-1α may be administered via a systemic administration or via a local administration.

As used herein the phrase "systemic administration" refers to oral, intravenous, intraperitoneal and intramuscular administration of SDF-1α of the present invention.

As used herein the phrase "local administering" refers to applying the SDF-1α of the present invention directly to the area of the implanted graft or in close proximity to the implanted graft. According to an exemplary embodiment, the SDF-1α of the present invention is directly administered to the transplanted cell, tissue or organ graft via injection.

It will be appreciated that according to the teachings of the present invention the contemplated dose of SDF-1α applied for local administration (e.g. for direct injection into the graft) may range between about 1 pg/kg to about 1 mg/kg, between about 0.01 ng/kg to about 1 mg/kg, between about 0.1 ng/kg to about 1 mg/kg, between about 1 pg/kg to about 0.1 mg/kg, between about 1 pg/kg to about 0.01 mg/kg, between about 1 pg/kg to about 0.001 mg/kg, between about 0.001 ng/kg to about 1000 ng/kg, between about 0.01 ng/kg to about 100 ng/kg, or between about 0.1 ng/kg to about 100 ng/kg of SDF-1α per kg body weight.

In cases of local administration into a population of cells (e.g. fat graft), the contemplated dose of SDF-1α may range between about 1 pg/1,000,000 cells to about 1 mg/1,000,000 cells, between about 0.01 ng/1,000,000 cells to about 1 mg/1,000,000 cells, between about 0.1 ng/1,000,000 cells to about 1 mg/1,000,000 cells, between about 1 pg/1,000,000 cells to about 0.1 mg/1,000,000 cells, between about 1 pg/1,000,000 cells to about 0.01 mg/1,000,000 cells, between about 1 pg/1,000,000 cells

to about 0.001 mg/1,000,000 cells, between about 0.001 ng/1,000,000 cells to about 1000 ng/1,000,000 cells, between about 0.01 ng/1,000,000 cells to about 100 ng/1,000,000 cells, or between about 0.1 ng/1,000,000 cells to about 100 ng/1,000,000 cells of SDF-1α per administration.

Likewise, the dose of SDF-1α for systemic administration may range between about 1 pg/kg to about 1 mg/kg, between about 0.01 ng/kg to about 1 mg/kg, between about 0.1 ng/kg to about 1 mg/kg, between about 1 pg/kg to about 0.1 mg/kg, between about 1 pg/kg to about 0.01 mg/kg, between about 1 pg/kg to about 0.001 mg/kg, between about 0.001 ng/kg to about 1000 ng/kg, between about 0.01 ng/kg to about 100 ng/kg, or between about 0.1 ng/kg to about 100 ng/kg of SDF-1α per kg body weight.

In cases of systemic administration for enhancing survival of a population of cells (e.g. fat graft), the contemplated dose of SDF-1α may range between about 1 pg/1,000,000 cells to about 1 mg/1,000,000 cells, between about 0.01 ng/1,000,000 cells to about 1 mg/1,000,000 cells, between about 0.1 ng/1,000,000 cells to about 1 mg/1,000,000 cells, between about 1 pg/1,000,000 cells to about 0.1 mg/1,000,000 cells, between about 1 pg/1,000,000 cells to about 0.01 mg/1,000,000 cells, between about 1 pg/1,000,000 cells to about 0.001 mg/1,000,000 cells, between about 0.001 ng/1,000,000 cells to about 1000 ng/1,000,000 cells, between about 0.01 ng/1,000,000 cells to about 100 ng/1,000,000 cells, or between about 0.1 ng/1,000,000 cells to about 100 ng/1,000,000 cells of SDF-1α per administration.

According to a specific embodiment, in cases of fat transplantation, the dose of SDF-1α for systemic or local administration may range between about 1 pg/ml to about 1 mg/ml, between about 0.01 ng/ml to about 1 mg/ml, between about 0.1 ng/ml to about 1 mg/ml, between about 1 pg/ml to about 0.1 mg/ml, between about 1 pg/ml to about 0.01 mg/ml, between about 1 pg/ml to about 0.001 mg/ml, between about 0.001 ng/ml to about 1000 ng/ml, between about 0.01 ng/ml to about 100 ng/ml, or between about 0.1 ng/ml to about 100 ng/ml of SDF-1α per ml fat per administration.

The dose of SDF-1α selected for treatment depends on the type of graft, on the subject being treated, on the location of the graft, and when transplanting cells, on the number and concentration of the cells.

It will be appreciated that when mimetics compositions are used the dosages of SDF-1α should be calibrated such as according to the molar value. Such a calibration is a routine calculation for those of ordinary skill in the art.

Administration of SDF-1α is typically effected immediately following implantation of the cell, tissue or organ graft. Thus, according to the present teachings, SDF-1α is administered to the subject within a few minutes or within a few hours of implantation. According to a specific embodiment, SDF-1α is administered to the subject starting from the first day of transplantation and is continuously administered until the graft has been integrated and vascularized in the subject (e.g. for at least about 1-10 days, 5-20 days, 10-40 days, 5-50 days or 1-90 days).

According to a specific embodiment, SDF-1α can be provided to the subject by daily injection or injections every 2 days. SDF-1α can also be also administered in slow release devices (e.g. scaffold or beads that slowly release SDF-1α). The release of SDF-1α is typically continued until optimal or acceptable results are reached (e.g. vascularization and integration of the graft). This can range from 1 day (i.e. a single administration), 1 month (about 10 administrations) or slow released for up to 1 month according to an acceptable dose.

According to another specific embodiment, SDF-1α is administered to the subject for about 1 day, about 3 days, about 5 days, about 1 week, about 2 weeks, about 1 month, about 2 months, about 3 months, about 6 months, about 1 year, about 1.5 years, about 2 years, about 2.5 years or about 3 years or more.

According to a specific embodiment, the present invention contemplates treating the cell, tissue or organ graft with SDF-1α prior to implantation thereof. This may be in addition to administration of SDF-1α following implantation or instead of administration of SDF-1α following implantation. Treatment of the cell, tissue or organ may be carried out by any method known to one of ordinary skill in the art as for example by *ex vivo* contacting the cell or tissue graft with SDF-1α in a tissue culture plate or by injection of SDF-1α directly into the tissue or organ graft. Alternatively, the cell, tissue or organ graft may be exposed to SDF-1α prior to removal from the donor.

Contemplated concentrations of SDF-1α for treating a cell, tissue or organ graft prior to transplantation (e.g. *ex vivo* or *in vivo*) include a dose between about 1 pg/1,000,000 cells to about 1 mg/1,000,000 cells, between about 0.01 ng/1,000,000 cells

to about 1 mg/1,000,000 cells, between about 0.1 ng/1,000,000 cells to about 1 mg/1,000,000 cells, between about 1 pg/1,000,000 cells to about 0.1 mg/1,000,000 cells, between about 1 pg/1,000,000 cells to about 0.01 mg/1,000,000 cells, between about 1 pg/1,000,000 cells to about 0.001 mg/1,000,000 cells, between about 0.001 ng/1,000,000 cells to about 1000 ng/1,000,000 cells, between about 0.01 ng/1,000,000 cells to about 100 ng/1,000,000 cells, or between about 0.1 ng/1,000,000 cells to about 100 ng/1,000,000 cells of SDF-1α per cells.

Moreover, contemplated concentrations of SDF-1α for treating a cell, tissue or organ graft prior to transplantation (e.g. *in vivo*) include a dose between about 1 pg/kg to about 1 mg/kg, between about 0.01 ng/kg to about 1 mg/kg, between about 0.1 ng/kg to about 1 mg/kg, between about 1 pg/kg to about 0.1 mg/kg, between about 1 pg/kg to about 0.01 mg/kg, between about 1 pg/kg to about 0.001 mg/kg, between about 0.001 ng/kg to about 1000 ng/kg, between about 0.01 ng/kg to about 100 ng/kg, or between about 0.1 ng/kg to about 100 ng/kg of SDF-1α per kg body weight.

According to a specific embodiment, in cases of fat transplantation, the contemplated concentrations of SDF-1α for treating a cell, tissue or organ graft prior to transplantation (e.g. *ex vivo* or *in vivo*) include a dose between about 1 pg/ml to about 1 mg/ml, between about 0.01 ng/ml to about 1 mg/ml, between about 0.1 ng/ml to about 1 mg/ml, between about 1 pg/ml to about 0.1 mg/ml, between about 1 pg/ml to about 0.01 mg/ml, between about 1 pg/ml to about 0.001 mg/ml, between about 0.001 ng/ml to about 1000 ng/ml, between about 0.01 ng/ml to about 100 ng/ml, or between about 0.1 ng/ml to about 100 ng/ml of SDF-1α per ml fat.

According to one embodiment, the subject may be treated with SDF-1α prior to implantation of the cell, tissue or organ graft. Such a subject may continue to receive SDF-1α following implantation of the graft as depicted in detail hereinabove.

According to another embodiment, SDF-1α may be administered to a subject who has previously received a cell, tissue or organ graft as to assist vascularization and/or integration thereof in the subject. In such cases, the subject may have undergone transplantation a few hours, a few days, a few weeks, a few months or a few years prior to administration of SDF-1α as detailed above.

According to an embodiment of the present invention, endothelial precursor cells (EPCs) are also administered to the subject.

As used herein, the term "endothelial precursor cells" (EPCs) refers to the stem cells capable of forming blood vessels (also known as endothelial progenitor cells). EPCs may be obtained by mobilization of same from the bone marrow using any mobilization factor known in the art (e.g. placental growth factor, G-CSF, GM-CSF and VEGF) and may be identified and selected by their cell surface expression of CD34+CD133+VEGFR2+[KDR+].

In accordance with the present methods, about 10,000 to about 10,000,000 cells/ml, about 10,000 to about 1,000,000 cells/ml, about 10,000 to about 100,000 cells/ml, about 1000 to about 10,000,000 cells/ml, about 100 to about 10,000,000 cells/ml, about 1000 to about 10,000 cells/ml or about 100 to about 1000 cells/ml of EPCs are typically administered to a subject.

EPCs according to the present methods may be autologous or non-autologous with respect to the subject as described in further detail hereinabove. Furthermore, EPCs may be co-administered with the cell, tissue or organ graft. Alternatively, EPCs may be administered independently from the graft, thus, EPCs may be administered to the subject prior to, concomitantly with or following transplantation of a cell, tissue or organ graft.

SDF-1α can be administered to the subject *per se* or as a pharmaceutical composition. In addition, the cell, tissue or organ graft of the present invention can be administered per se or as part of a pharmaceutical composition.

As used herein a "pharmaceutical composition" refers to a preparation of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of the composition is to facilitate administration of the active ingredients (e.g., SDF-1α) to the subject.

Herein the term "active ingredient" refers to the agent accountable for the biological effect.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to the subject and does not abrogate the biological activity and properties of the administered active ingredients. An adjuvant is included under these phrases.

Herein, the term "excipient" refers to an inert substance added to the composition (pharmaceutical composition) to further facilitate administration of an active ingredient of the present invention.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

As mentioned hereinabove, suitable routes of administration of SDF-1α may, for example, include a systemic manner including oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intravenous, inrtaperitoneal, intramuscular, intranasal, or intraocular injections.

Alternately, one may administer the pharmaceutical composition comprising SDF-1α in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into the cell, tissue or organ region of a patient, or via application of the compositions directly into a tissue region in proximity to the implant area of a patient. Suitable routes of administration of the compositions may, for example, include topical (e.g., to a keratinous tissue, such as the skin, scalp) and mucosal (e.g., oral, vaginal, eye) administrations.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the pharmaceutical composition to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to the present invention are conveniently delivered in the form of an aerosol spray

presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continues infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The pharmaceutical composition of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients (e.g. SDF-1α) effective in enhancing a cell, tissue or organ graft survival or enhancing transplantation thereof.

According to an embodiment, a therapeutically effective amount is for promoting angiogenesis and reducing apoptosis of the cell, tissue or organ graft.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from in vitro and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental animals. The data obtained from these in vitro and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to levels of the active ingredient which are sufficient to induce or suppress the biological effect (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

An animal model which can be used according to the present teachings to assess the biological effect of the compositions described herein includes diabetic nude mice (as described in detail in Example 1 of the Examples section which follows).

Depending on the severity of the condition being treated, on the type of cell, tissue or organ graft and the responsiveness of the subject to the treatment, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or months or until cure is effected or until the cell, tissue or organ graft has been acceptably integrated in the subject.

According to some embodiments of the present invention, SDF-1α compositions are administered once, administered twice, administered three times, administered four times, administered five times, administered six times, administered seven times, administered eight times, administered nine times or administered ten times to the subject in order to enhance survival of the cell, tissue or organ graft. It will be appreciated that if multiple transplantations are carried out (e.g. fat cell transplantations), the number of administrations of SDF-1α may be vast and may be prolonged for as long as needed (as determined by one of ordinary skill in the art). Preferably, the compositions of the present invention are administered at least once a day. It will be appreciated that the number of administrations can be determined by one of ordinary skill in the art.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Determination of efficacy of treatment may be determined by measuring the number and viability of the engrafted cells (e.g. by ultrasound), measuring the number of apoptotic cells within the graft (e.g. by PCR), and evaluating the vascularization of the transplanted cells, tissues or organs (e.g. by ultrasound).

Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as an FDA-approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser device may also be accompanied by a notice in a form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions for human or veterinary administration. Such notice, for example, may include labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a pharmaceutically acceptable carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition, as further detailed above.

It will be appreciated that the SDF-1α and the cell, tissue or organ graft (e.g. fat cells) may be formulated in a single pack or alternatively may be placed in separate packs.

Since the compositions of the present invention are utilized *in vivo*, the compositions are preferably of high purity and substantially free of potentially harmful contaminants, e.g., at least National Food (NF) grade, generally at least analytical grade, and preferably at least pharmaceutical grade. To the extent that a given compound must be synthesized prior to use, such synthesis or subsequent purification shall preferably result in a product that is substantially free of any potentially contaminating toxic agents that may have been used during the synthesis or purification procedures.

Additional factors may be incorporated into the compositions of the present invention (*i.e.*, SDF-1α described hereinabove) to enhance survival of the cell, tissue or organ graft or to enhance transplantation thereof. These include, but are not limited to, extracellular matrix components (e.g. vitronectin, laminin, collagen, elastin), growth factors (e.g. FGF 1, FGF 2, IGF 1, IGF 2, PDGF, EGF, KGF, HGF, VEGF, GM-CSF, CSF, G-CSF, TGF alpha, TGF beta, NGF and ECGF), hypoxia inducible factors (e.g. HIF-1 alpha and beta and HIF-2), hormones (e.g., erythropoietin, insulin, growth hormone (GH), CRH, Leptin, Prolactin and TSH), angiogenic factors (e.g., angiogenin and angiopoietin), coagulation and anticoagulation factors [e.g., Factor I, Factor XIII, tissue factor, calcium, vWF, protein C, protein S, protein Z, fibronectin, antithrombin, heparin, plasminogen, low molecular weight heparin (Clixan), high molecular weight kininogen (HMWK), prekallikrein, plasminogen activator inhibitor-1 (PAI1), plasminogen activator inhibitor-2 (PAI2), urokinase, thrombomoduline, tissue plasminogen activator (tPA), alpha 2-antiplasmin and Protein Z-related protease inhibitor (ZPI)], cytokines (IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13 and INF-alpha, INF, beta, and INF-gamma), chemokines (e.g., MCP-1 or CCL2), enzymes (e.g. endoglycosidases, exoglycosidases, endonucleases, exonucleases, peptidases, lipases, oxidases, decarboxylases, hydrases, chondroitinase, chondroitinase ABC, chondroitinase AC, hyaluronidase, keratanase, heparanases, heparanase splice variance, collagenase, trypsin, catalases), neurotransmitters (e.g., acetylcholine and monoamines), neuropeptides (e.g. substance P), vitamins (e.g., D-biotin, Choline Chloride, Folic acid, Myo-inositol, Niacinamide, D-

Pantothenic acid, Calcium salts, Pyridoxal.HCl, Pyrodixine.HCl, Riboflavin, Thiamine.HCl, Vitamin B12, vitamin E, vitamin C, vitamin D, vitamin B1-6, vitamin K, vitamin A and vitamin PP), carbohydrates (e.g. Mono/Di/Polysacharides including glucose, mannose, maltose and fructose), ions, chelators (e.g. Fe chelators, Ca chelators), antioxidants (e.g., Vitamin E, Quarcetin, superoxide scavengers, Superoxide dismutase), H2O2 scavengers, free radicals scavengers, Fe scavengers), fatty acids (e.g., Triglycerides, Phospholipids, Cholesterols, free fatty acids and non free fatty acids, fatty alcohol, Linoleic acid, oleic acid and lipoic acid), antibiotics (e.g., Penicillins, Cephalosporins and Tetracyclines), analgesics, anesthetics, antibacterial agents, anti-yeast agents, anti-fungal agents, antiviral agents, pro-biotic agents, anti-protozal agents, anti-pruritic agents, anti-dermatitis agents, anti-emetics, anti-inflammatory agents, anti-hyperkeratolyic agents, antiperspirants, anti-psoriatic agents, anti-seborrheic agents, antihistamine agents, amino acids (e.g., essential and non essential (from A–Z) especially glutamine and arginine), salts (e.g., prurivat salts and sulfate salts), sulfates (e.g. Calcium Sulfate), steroids (e.g., androgens, estrogens, progestagens, glucocorticoids and mineralocorticoids), catecholamines (e.g., Epinephrine and Nor-epinephrine), Nucleosides and Nucleotides (e.g., Purins and Pyrimidines), Prostaglandins (e.g. Prostaglandin E2), Leucotriens, Proteoglycans (e.g. Heparan sulfate, keratan sulfate), Hydroxyapatites [e.g. Hydroxyapatite $(Ca_{10}(PO_4)_6(OH)_2)$], Haptoglobins (Hp1-1, Hp2-2 and Hp1-2), Superoxide dismutases (e.g. SOD 1/2/3), Nitric Oxides, Nitric Oxide donors (e.g. nitroprusside, Sigma Aldrich, St. Louis, MO, USA, Glutathione peroxidases, Hydrating compounds (e.g. vasopressin), cells (e.g. Platelets), cell medium (e.g. M199, DMEM/F12, RPMI, Iscovs), serum (e.g. human serum, fetal calf serum, , fetal bovine serum), buffers (e.g., HEPES, Sodium Bicarbonate), detergents (e.g., Tween), disinfectants, herbs, fruit extracts, vegetable extracts (e.g. cabbage, cucumber), flower extracts, plant extracts, flavinoids (e.g. pomegranate juice), spices, leafs (e.g. Green tea, Chamomile), Polyphenols (e.g. Red Wine), honey, lectins, microparticles, nanoparticles (lyposomes), micelles, calcium carbonate (CaCO3, e.g. precipitated calcium carbonate, ground/pulverized calcium carbonate, albacar, PCC, GCC), calcite, limestone, crushed marble, ground limestone, lime, chalk (e.g. whiting chalk, champagne chalk, french chalk) and co factors such as BH4 (tetrahydrobiobterine).

The present composition may also contain ingredients, substances, elements and materials containing, hydrogen, alkyl groups, aryl groups, halo groups, hydroxy groups, alkoxy groups, alkylamino groups, dialkylamino groups, acyl groups, carboxyl groups, carboamido groups, sulfonamide groups, aminoacyl groups, amide groups, amine groups, nitro groups, organo selenium compounds, hydrocarbons, and cyclic hydrocarbons.

The present composition may be combined with substances such as benzol peroxide, vasoconstrictors, vasodilatators, salicylic acid, retinoic acid, azelaic acid, lactic acid, glycolic acid, pyreuric acid, tannins, benzlidenecamphor and derivatives thereof, alpha hydroxyis, surfactants.

Compositions of some embodiments of the present invention may be bioconjugated to polyethylenglycol (e.g. PEG, SE-PEG) which preserves the stability (e.g., against protease activities) and/or solubility (e.g., within a biological fluid such as blood, digestive fluid) of the active ingredients (e.g. SDF-1α) while preserving their biological activity and prolonging its half-life.

It will be appreciated that compositions of the present invention can be used in combination with other currently practiced therapies for cell, tissue or organ transplantation as, without being limited to, treatment of the subject with growth factors, transplantation of the cells or tissues on scaffolds or transplantation of the cells on polyester beads.

As mentioned, the cell, tissue or organ graft of the present invention can be derived from either autologous sources or from non-autologous sources (e.g. allogeneic or xenogeneic). Since non-autologous cells, tissues or organs are likely to induce an immune reaction when administered to the body several approaches have been developed to reduce the likelihood of rejection of non-autologous grafts. These include either suppressing the recipient immune system or encapsulating the non-autologous grafts (e.g. cells or tissues) in immunoisolating, semipermeable membranes before transplantation.

Encapsulation techniques are generally classified as microencapsulation, involving small spherical vehicles and macroencapsulation, involving larger flat-sheet and hollow-fiber membranes (Uludag, H. et al. Technology of mammalian cell encapsulation. Adv Drug Deliv Rev. 2000; 42: 29-64).

Methods of preparing microcapsules are known in the arts and include for example those disclosed by Lu MZ, et al., Cell encapsulation with alginate and alpha-phenoxycinnamylidene-acetylated poly(allylamine). Biotechnol Bioeng. 2000, 70: 479-83, Chang TM and Prakash S. Procedures for microencapsulation of enzymes, cells and genetically engineered microorganisms. Mol Biotechnol. 2001, 17: 249-60, and Lu MZ, et al., A novel cell encapsulation method using photosensitive poly(allylamine alpha-cyanocinnamylideneacetate). J Microencapsul. 2000, 17: 245-51.

For example, microcapsules are prepared by complexing modified collagen with a ter-polymer shell of 2-hydroxyethyl methylacrylate (HEMA), methacrylic acid (MAA) and methyl methacrylate (MMA), resulting in a capsule thickness of 2-5 μm. Such microcapsules can be further encapsulated with additional 2-5 μm ter-polymer shells in order to impart a negatively charged smooth surface and to minimize plasma protein absorption (Chia, S.M. et al. Multi-layered microcapsules for cell encapsulation Biomaterials. 2002 23: 849-56).

Other microcapsules are based on alginate, a marine polysaccharide (Sambanis, A. Encapsulated islets in diabetes treatment. Diabetes Thechnol. Ther. 2003, 5: 665-8) or its derivatives. For example, microcapsules can be prepared by the polyelectrolyte complexation between the polyanions sodium alginate and sodium cellulose sulphate with the polycation poly(methylene-co-guanidine) hydrochloride in the presence of calcium chloride.

It will be appreciated that cell encapsulation is improved when smaller capsules are used. Thus, the quality control, mechanical stability, diffusion properties, and in vitro activities of encapsulated cells improved when the capsule size was reduced from 1 mm to 400 μm (Canaple L. et al., Improving cell encapsulation through size control. J Biomater Sci Polym Ed. 2002;13: 783-96). Moreover, nanoporous biocapsules with well-controlled pore size as small as 7 nm, tailored surface chemistries and precise microarchitectures were found to successfully immunoisolate microenvironments for cells (Williams D. Small is beautiful: microparticle and nanoparticle technology in medical devices. Med Device Technol. 1999, 10: 6-9; Desai, T.A. Microfabrication technology for pancreatic cell encapsulation. Expert Opin Biol Ther. 2002, 2: 633-46).

As mentioned above, in order to facilitate engraftment of non-autologous grafts, the method of the present invention may further advantageously comprise conditioning

the subject with an immunosuppressive regimen prior to, concomitantly with, or following transplantation of the cell, tissue or organ graft.

According to a specific embodiment, the methods of the present invention require a reduced immunosuppressive regimen as compared to a subject not treated with SDF-1α.

Examples of suitable types of immunosuppressive regimens include administration of immunosuppressive drugs and/or immunosuppressive irradiation.

Ample guidance for selecting and administering suitable immunosuppressive regimens for transplantation is provided in the literature of the art (for example, refer to: Kirkpatrick CH. and Rowlands DT Jr., 1992. JAMA. 268, 2952; Higgins RM. et al., 1996. Lancet 348, 1208; Suthanthiran M. and Strom TB., 1996. New Engl. J. Med. 331, 365; Midthun DE. et al., 1997. Mayo Clin Proc. 72, 175; Morrison VA. et al., 1994. Am J Med. 97, 14; Hanto DW., 1995. Annu Rev Med. 46, 381; Senderowicz AM. et al., 1997. Ann Intern Med. 126, 882; Vincenti F. et al., 1998. New Engl. J. Med. 338, 161; Dantal J. et al. 1998. Lancet 351, 623).

Preferably, the immunosuppressive regimen consists of administering at least one immunosuppressant agent to the subject.

Examples of immunosuppressive agents include, but are not limited to, methotrexate, cyclophosphamide, cyclosporine, cyclosporin A, chloroquine, hydroxychloroquine, sulfasalazine (sulphasalazopyrine), gold salts, D-penicillamine, leflunomide, azathioprine, anakinra, infliximab (REMICADE), etanercept, TNF.alpha. blockers, a biological agent that targets an inflammatory cytokine, and Non-Steroidal Anti-Inflammatory Drug (NSAIDs). Examples of NSAIDs include, but are not limited to acetyl salicylic acid, choline magnesium salicylate, diflunisal, magnesium salicylate, salsalate, sodium salicylate, diclofenac, etodolac, fenoprofen, flurbiprofen, indomethacin, ketoprofen, ketorolac, meclofenamate, naproxen, nabumetone, phenylbutazone, piroxicam, sulindac, tolmetin, acetaminophen, ibuprofen, Cox-2 inhibitors and tramadol. These agents may be administered individually or in combination.

According to another embodiment, the methods of the present invention require a reduced anti-inflammatory treatment [e.g. anti-inflammatory drugs such as steroids, non-

steroidal anti-inflammatory drugs or immune selective anti-inflammatory derivatives (ImSAIDs)] as compared to a subject not treated with SDF-1α.

As illustrated in the Examples section which follows, the present inventors have further shown that transplantation of a graft (e.g. fat graft) along with SDF-1α treatment leads to a suppressed graft rejection and/or graft versus host disease (GVHD) as illustrated by a higher weight and volume of the transplanted graft as compared to a graft transplanted without SDF-1α treatment.

Thus, according to an embodiment of the present invention, there is provided a method of preventing graft rejection or graft versus host disease of a cell, tissue or organ graft in a subject in need thereof, the method comprising (a) implanting a cell, a tissue or an organ graft into the subject; and prior to, concomitantly with, or following (b) administering to the subject a therapeutically effective amount of SDF-1α.

Methods of assessing graft rejection are well known in the art and include, for example, monitoring the subject for symptoms such as less urine output (e.g. for kidney transplants), yellow skin color and easy bleeding (e.g. for liver transplants) and shortness of breath and less tolerance to exertion (e.g. for heart transplants). Additional methods include e.g. biopsy of the transplanted organ, lab tests of e.g. kidney or liver function, ultrasound of the transplanted graft, arteriography (e.g. kidney), CT scan, heart echocardiography, chest x-ray and MRI.

Likewise, methods of assessing GVHD are well known in the art and include, for example, monitoring the subject for symptoms such as abdominal pain or cramps, diarrhea, fever, jaundice, skin rash, vomiting and weight loss. Additional methods include e.g. immunohistochemical and FISH analyses.

As used herein the term "about" refers to ± 10 %.

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of means "including and limited to".

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

As used herein, the term "treating" includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided

separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and

Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1

Efficient fat transplantation with local SDF1α treatment

MATERIALS AND EXPERIMENTAL PROCEDURES

Isolation of human fat tissue

Fat was harvested from the thigh of a 40-year-old woman by suction-assisted lipectomy under general anesthesia. Before the beginning of the procedure, a local anesthesia solution containing lidocaine (0.5 %) and adrenaline (1:1,000,000) was injected to the areas designated for aspiration in order to decrease bleeding during the procedure and to relieve pain after it. The fat was aspirated using a 14-gauge blunt cannula with three holes near its tip that was attached to a sterile 10 cc syringe. The aspirated fat was then processed and prepared under sterile conditions for subsequent grafting into nude mice within two hours of its collection according to previously published protocols [Ullmann Y et al., Dermatol Surg (2005) 31:1304-7]. The procedure was reviewed and approved by the Institutional Review Board of the Rambam Health Care Campus, and the donor gave her written informed consent.

Isolation, cultivation and characterization of endothelial progenitor cells (EPCs)

All experimental procedures were reviewed and approved by the Technion Animal Care and Use Committee. EPCs were isolated from bone marrow (BM) of adult eight-week-old female CD-1 nude mice (n=20) and then cultured according to a

previously described protocol [Wang et al., J Cell Biochem. (2008) 103(1):21-9]. In brief, BM mononuclear cells (BM-MNCs) were isolated by density gradient centrifugation using Lymphoprep™ (Axis-Shield, Oslo, Norway), and then grown in endothelial cell basal medium-2 (EBM-2) (PromoCell GmbH, Heidelberg, Germany) at 37 °C / 5 % CO_2 for four days. The EPCs were identified as adherent cells that stained double positive for acetylated LDL (acLDL) and lectin under a fluorescent microscope (Vasa M. 2001). For this purpose, the adherent cells from the EPC cultures were first incubated with 1,1'-dioctadecyl-3,3,3',3'–tetramethylindocarbocyanine-labeled acLDL (DiI-acLDL; Biomedical Technologies), fixed in 2 % paraformaldehyde, and then counterstained with fluorescein isothiocyanate-labeled lectin from Bandeiraea Simplicifolia (BS-1) (Sigma Aldrich, MO, USA). For further characterization, the putative EPCs were immunostained for (a) CD34, (b) stem cell antigen-1 (Sca-1) (c) fetal liver kinase-1 (Flk-1), and (d) CD31 using monoclonal antibodies all of which were purchased from Santa Cruz, CA, USA.

Study design

The study comprised eighty adult eight-week-old female CD-1 nude mice (Harlan, Jerusalem, Israel). The mice were acclimated for one week prior to the study in a room with an artificial 12 h light/dark cycle, and were fed a standard laboratory chow and water ad libitum. Diabetes was then induced in sixty mice by six intraperitoneal injections of 10 mg/kg/injection of streptozotocin (STZ) (Sigma Aldrich, MO, USA) repeated every other two days for each mouse. The blood glucose level in a drop of tail blood from each STZ-injected mouse was measured by a glucometer (FreeStyle, Alameda, CA, USA), and mice were considered diabetic when their blood glucose levels were greater than 300 mg/dl. Twenty apparently healthy mice were used for the non-diabetic groups.

Ten days after the last STZ injection, all mice received a transplant of 1 ml aspirated human fat. The fat was injected subcutaneously into the scalp using a 14 G needle while the animals were manually restrained. Immediately following fat injection, the fat grafts of the non-diabetic mice were treated either with 100 µl phosphate buffer saline (PBS-treated non-diabetic control group; n=10) or with 25 µg/kg SDF1α [recombinant human SDF1, PeproTech] in 100 µl PBS (SDF1α-treated non-diabetic group; n=10). The diabetic mice were randomly divided into six groups according to

treatment of the transplanted human fat subsequent to its injection into the scalps of mice. The fat grafts of the diabetic mice were treated with either 100 µl PBS (PBS-treated diabetic control group; n=10), 10^6 $Sca1^+/Flk1^+$ cells in 100 µl EBM-2 (EPC-treated diabetic group; n=10), or 25 µg/kg SDF1α in 100 µl PBS (n=40) of which 10 mice were used as the SDF1α-treated diabetic group (SDF1α group; n=10), 10 mice were treated intraperitoneally (i.p.) with 7.5 mg/kg AMD3100 in 100 µl PBS (SDF1α/AMD3100-treated group; n=10), 10 mice were treated intraperitoneally with 20 mg/kg L-NAME in 100 µl PBS (SDF1α/L-NAME-treated group; n=10), and 10 mice were treated intraperitoneally with 50 µg/kg anti-SDF1α antibody in 100 µl PBS (SDF1α/anti-SDF1α antibody group; n=10). All injections were repeated every three days for 18 days. SDF1α, AMD3100, L-NAME and anti-SDF1α antibody were all purchased from Sigma. No post-procedural treatment of the non-diabetic or diabetic mice was performed. Post-operative analgesics and antibiotics were not administered to the mice.

Follow-up and data collection

The duration of the study period was 15 weeks after fat transplantation, and during the study period, each mouse was weighed 3 times; on the first day, on day 18 and on the last day of the experiment. The mice were maintained under the same conditions as described in the "study design" section above. After 15 weeks, all mice were sacrificed humanely by an overdose of ketamine. After death, the fat grafts were carefully dissected from the scalps of the mice and then weighed. The volume of each of the fat grafts was measured by the liquid overflow method [Ayhan M. et al., Aesthetic Plast Surg (2001) 25: 338-342]. For this purpose, the fat graft was placed in a 15-ml sterile measuring cylinder that contained 10 cc sterile PBS. The volume of the fat graft was considered to be 1 ml when the level of the solution rose to the 11 cc mark. After weight and volume determination, each fat graft was divided into two portions from the middle. One portion was stored at -80 °C until required for determining the expression levels of VEGF receptor-2 (VEGF-R2), CXCR4, eNOS and AKT and the apoptotic protein levels of caspase-3 and cytochrome c by western blot (see further detail hereinbelow). The second portion was placed in 4 % formalin for histological examination and determining microvascular density (MVD).

To investigate whether SDF1α affects fat graft survival through an EPC-mediated angiogenesis mechanism at an early period after fat transplantation, some mice were sacrificed humanely by an overdose of ketamine 18 days after fat transplantation (n=4 for each group). After death, the fat grafts were carefully dissected from the scalps of the mice, and homogenized to determine the expression levels of VEGF-R2, CXCR4, eNOS and AKT by western blot (see further detail hereinbelow). At each time point, 0.5 ml blood was collected from the heart of each mouse immediately before sacrifice to determine the red blood cell, leukocyte and platelet counts, and the plasma hemoglobin, plasma SDF1α and VEGF concentrations. In addition, the frequency of EPCs in circulating peripheral blood mononuclear cells (PB-MNCs) and in BM-MNCs was assessed using flow cytometry by determining the frequency of Sca-1 and Flk-1 positive cells (see below).

Flow Cytometry

PB-MNCs and BM-MNCs obtained before fat transplantation and on day 18 and week 15 after fat transplantation were isolated and cultured with EBM-2 at 37 °C. After 4 days, EPC populations in BM-MNCs and PB-MNCs were further analyzed using fluorescence-activated cell sorting (FACS) with phycoerythrin (PE)-conjugated antibody against Sca-1 and fluorescein isothiocyanate (FITC)-conjugated antibody against Flk-1 antigens (eBioscience, San Diego, CA, USA) as previously described [Goldstein LJ et al., Stem Cells (2006) 24(10):2309-18]. Isotype-matched IgGs were used as negative controls. Immunofluorescence-labeled cells were analyzed by the Macintosh Cell-Quest Software program counting 100,000 events per sample (Becton Dickinson, NJ, USA).

ELISA

Plasma SDF1α and VEGF levels were determined using a commercial enzyme-linked immunosorbent assay (ELISA) Quantikine IVD® SDF1α Kit and Quantikine IVD® VEGF Kit both from (R&D Systems, MN, USA) in accordance with the manufacturer's instructions, and were expressed as picograms per ml.

Histological analysis

Histological slides of the formalin-maintained samples were prepared from fat grafts of sacrificed mice at week 15, and then stained with hematoxylin and eosin using standard procedures. To this end, the formalin-maintained samples were washed in tap water, dehydrated using an ascending alcohol series, and then embedded in paraffin

blocks. Sections (5 μm thick) were cut, mounted on glass slides, and then hydrated using distilled water. The slides were examined under a light microscope for (a) the extent of integration, as evidenced by the extent of organization of intact and nucleated fat cells, (b) the extent of fibrosis, as evidenced by the amount of collagen and elastic fibrils, (c) the presence of cysts and vacuoles, and (d) the intensity of the inflammatory response, as evidenced by the extent of lymphocyte and macrophage infiltration. Each criterion was graded on a scale of 0 to 5 where 0 = absence, 1 = minimal presence, 2 = minimal to moderate presence, 3 = moderate presence, 4 = moderate to extensive presence, and 5 = extensive presence. All evaluations were made by investigators who were blind to the treatment of the mice.

Assessment of microvascular density (MVD)

The paraffin-embedded fat graft sections were incubated with a monoclonal antibody against tissue CD31 (R&D Systems, Minneapolis MN, USA) overnight at room temperature. Upon completion of the incubation, the specimens were counterstained with hematoxylin. Mouse IgG was used as a negative control. The microvascular density (MVD) was determined in five regions of interest where the CD31 antibody signal was the most intense. The number of blood vessels in each region was counted under a light microscope at 40 x magnification by investigators who were blind to the treatment of the mice.

Western blotting

The expression levels of VEGF-R2, CXCR4, eNOS and AKT, and the apoptotic proteins caspase 3 and cytochrome c were determined by western blotting in homogenates of the harvested fat grafts on day 18. Homogenates of samples of the fat grafts were lysed in RIPA buffer (R&D Systems, MN, USA). A 40 μg aliquot of each lysate was loaded onto SDS-PAGE, and then transferred to nitrocellulose membranes. Membranes were then incubated with monoclonal antibodies against VEGF-R2, CXCR4, eNOS and AKT, and caspase 3, and cytochrome c (all purchased from Santa Cruz, CA, USA), before a second incubation with a horseradish peroxidase (HRP)-conjugated IgG secondary antibody. An antibody against β-actin (Santa Cruz) was used to normalize protein loading. The resultant bands were quantified by densitometry.

EPC Migration Assay

Migration of EPCs that were obtained from PB-MNCs of non-diabetic and diabetic mice was assessed at day 18 and week 15, after 4 days in culture. To this end, inventors used a Boyden chamber-based cell migration assay chamber using the CytoSelect® 96-well Cell Migration Assay (Cell Biolabs, CA, USA) in accordance with the manufacturer's protocol. In brief, the feeder trays were coated with 100 µl of EBM-2 that contained 200 ng/ml) of recombinant human SDF1α (Sigma Aldrich, MO, USA). An aliquot of EPCs (100,000 cells) in 100 µl serum-free EBM-2 was added to each well, and then incubated with the feeder tray for 12 hours at 37 °C. The migratory EPCs were detached from the membrane by cell detachment solution, and then lysed with a fluorescent dye-containing lysis buffer. The extent of migration was assessed by the intensity of the fluorescence signal which was measured in a fluorescence spectrophotometer. The results were expressed as the average percentage of relative fluorescence units (RFU) of cultured non-treated, non-diabetic EPCs in duplicates, or cultured control EPCs from four independent experiments.

In vitro tube formation on matrigel assay

Other EPCs that were obtained from PB-MNCs of non-treated, non-diabetic mice were incubated in high glucose concentrations (25 mmol/l) for 4 days. After 4 days, glucose-stressed EPCs or non-stressed EPCs (control) were detached using trypsin EDTA (0.5 %) and then cultured onto matrigel coated 96-well tissue culture plates supplemented with SDF1α. To this end, frozen matrigel (Sigma Aldrich, St Louis MO, USA) was thawed, supplemented with 200 ng/ml SDF1α and spread onto 96-well plates (50 µl/well) at room temperature for 30 minutes to allow solidification. The detached EPCs (5 x 10^4 cells/100µl EBM-2/well) were placed on the matrigel surface, and then incubated at 37 °C for 24 hours in EBM-2. After plating on the matrigel, EPCs were treated either with anti-SDF1α antibody (10 ng/ml), AMD3100 (2 µg/ml) or L-NAME (100 µg/ml). After 24 hours, the non-integrated cells were removed by washing, and tube formation on matrigel was assessed under a light microscope at x10 magnification. The tubular structures were graded semiquantitatively by evaluating the presence and stages of tube formation on a scale of 0 to 5: 0 = well separated individual cells, 1 = cells had begun to migrate and align themselves, 2 = visible capillary tubes and no sprouting, 3 = visible sprouting of new capillary tubes, 4 = early formation of closed polygons, 5 =

development of complex mesh-like structures. All evaluations were assessed by investigators who were blind to the treatments. Four random high-power fields in each sample were examined. The results from each examiner were then pooled in order to calculate the mean value for each criterion for each sample in each group.

Data analysis

Data for each study parameter from the PBS-, EPCs- and the SDF1α-treated fat grafts were pooled, and the results were presented as mean ± standard deviation (SD). The data presented a normal distribution by the Kolmogorov-Smirnov test. The data were statistically analyzed by one-way analysis of variance using a computerized statistical software program (Prism version 5.0, GraphPad Software Inc, CA, USA). Differences were considered statistically significant when $P \leq 0.05$. Kappa values for intra-examiner repeatability of the blinded evaluations of histological analysis, and MVD were 0.92 and 0.91, respectively.

RESULTS

All of the non-diabetic mice completed the 15-week study period. Five diabetic mice died: one mouse each from the PBS-treated, EPC-treated, SDF1α/L-NAME-treated, SDF1α-treated and SDF1α/anti-SDF1α antibody-treated fat graft groups. The red blood cell, leukocyte and platelet counts, and the plasma hemoglobin and SDF1α concentrations in the PBS-treated non-diabetic and diabetic groups, and the EPC-treated diabetic group did not change over the 15 week study period. In the SDF1α-treated fat grafts in the non-diabetic group and SDF1α-treated fat grafts in the diabetic group, only the leukocyte counts significantly increased (Table 1, below). On day 18, plasma SDF1α and VEGF concentrations increased significantly in diabetic and non-diabetic mice whose fat grafts were treated with SDF1α compared to other mice whose fat grafts were not treated with SDF1α. At week 15, plasma SDF1α concentrations in diabetic and non-diabetic mice whose fat grafts were treated with SDF1α returned to levels found in other mice whose fat grafts were not treated with SDF1α. However, plasma VEGF concentrations remained higher in diabetic and non-diabetic mice whose fat grafts were treated with SDF1α compared to other mice whose fat grafts were not treated with SDF1α. Tissue VEGF concentrations increased significantly in SDF1α-treated fat grafts in non-diabetic mice and in EPC- and SDF1α-treated fat grafts in diabetic mice compared to other treatments (Table 1, below).

Table 1

	Non Diabetic Control N=10			*Non Diabetic SDF1α N=10*			*Diabetic Control N=10*			*Diabetic EPC N=10*			*Diabetic SDF1α N=10*			*P Value (Between Groups After Treatment)*
Day (d)	**d0**	**d18**	**w15**	**d0**	**d18**	**w15**	**d0**	**d18**	**w15**	**d0**	**d18**	**w15**	**d0**	**d18**	**w15**	**d18 / w15**
N	*10*	*4*	*6*	*10*	*4*	*6*	*10*	*4*	*5*	*10*	*4*	*5*	*10*	*4*	*5*	
Body weight *(g)*	**27±1**	**28±1**	**28±1**	**27±1**	**27±1**	**28±1**	**26±1**	**25±1**	**24±2 ***	**27±1**	**26±1**	**24±1 ***	**27±1**	**26±1**	**24±1 ***	*NS / <0.05*
Blood glucose levels *(mg/dl)*	**89±3**	**85±3**	**92±4**	**90±4**	**89±4**	**94±4**	**474± 49**	**483± 55**	**422± 56**	**431± 50**	**398± 60**	**432± 58**	**427± 52**	**391± 49**	**446± 58**	*<0.001 / <0.001*
RBC count *($10^6/mm^3$)*	**7.7±0 .3**	**7.6±0 .4**	**7.7±0 .4**	**7.6±0 .7**	**7.9±0 .4**	**7.1±0 .3**	**7.2±0 .2**	**7.0±0 .4**	**7.0±0 .4**	**7.2±0 .6**	**7.9±0 .3**	**7.6±0 .5**	**7.2±0 .4**	**7.3±0 .4**	**7.0±0 .4**	*NS / NS*

	Non Diabetic Control N=10			*Non Diabetic SDF1α N=10*			*Diabetic Control N=10*			*Diabetic EPC N=10*			*Diabetic SDF1α N=10*			*P Value (Between Groups After Treatment)*
Leukocyte count *($10^6/mm^3$)*	10±0.8	10±1.0	10.2±0.8	10.1±1.1	14.1±1.7*	10.2±1.2	10.3±1.0	10.6±0.7	10.8±0.9	10.6±1.1	10.3±0.8	10.1±1.0	9.9±0.8	13.6±1.6*	10.2±0.9	*<0.001 / NS*
Platelet count *($10^3/L$)*	362±72	392±87	373±85	378±76	392±92	409±89	374±83	391±74	380±82	382±76	399±82	405±91	397±81	401±96	411±98	*NS / NS*
Hemoglobin levels *(g/dl)*	12.1±1.0	11.8±1.0	12.1±0.9	12.2±1.1	11.9±1.1	11.9±1.0	12.0±1.0	11.8±0.9	11.7±1.1	12.2±1.0	12.3±1.1	12.0±1.0	11.8±0.9	12.0±1.0	12.1±1.2	*NS / NS*
Plasma SDF1α concentration (ng/mL)	0.72±0.2	0.70±0.2	0.68±0.2	0.66±0.2	54.9±5.3**	0.68±0.2	0.73±0.3	0.72±0.3	0.74±0.4	0.70±0.3	0.75±0.4	0.74±0.4	0.71±0.2	62.5±6.6**	0.77±0.4	*<0.001 / NS*
Plasma VEGF concentration (pg/mL)	72±4	69±5	70 ±4	72±4	243±38 **	104±8*	71±5	74±4	89±6	67±3	74±4	88±6	66±4	288±46**	95±7*	*<0.001 / <0.05*

	Non Diabetic Control N=10			*Non Diabetic SDF1α N=10*			*Diabetic Control N=10*			*Diabetic EPC N=10*			*Diabetic SDF1α N=10*			*P Value (Between Groups After Treatment)*
Tissue VEGF concentration (ng/mL)	---	**0.2±0 .02**	**0.3±0 .02**	---	**1.4±0 .3 ****	**0.6±0 .06 ****	---	**0.3±0 .05**	---	---	**0.4±0 .04 ***	**0.4±0 .03 ***	---	**1.6±0 .4 ****	**0.5±0 .06 ***	*<0.001 / <0.05*

Table 1: Values are presented as mean ± SD. (N) Number of mice. Statistical significance is set at P<0.05. *P<0.05, **P<0.001 indicate difference from non diabetic mice whose fat graft was treated with phosphate buffered saline (PBS, control); RBC, red blood cells; SDF1α, stromal derived factor-1 alpha.

Fat weight and volume

Complete resorption of the fat grafts in all the PBS-treated diabetic mice and SDF1α/anti-SDF1α antibody-treated mice was observed 15 weeks after fat transplantation, although connective tissue devoid of fat was found. In some diabetic mice that were treated with SDF1α/AMD3100 and SDF1α/L-NAME, however, inventors found very small fat grafts. In contrast, the fats grafts were successful in the PBS- and SDF1α-treated grafts of non-diabetic mice, and in the EPC- and SDF1α-treated fat grafts in the diabetic mice, as evidenced by well-defined subcutaneous lumps on the animal's scalps (Figures 1A-L), and subsequently from measuring the isolated fat grafts (Figures 1M-T). The weight and volume of the SDF1α-treated fat grafts in the non-diabetic mice were significantly higher than those in the PBS-treated non-diabetic mice (Figures 2A and 2C). The weight and volume of the EPC-treated fat grafts in the diabetic mice were not different from those in the PBS-treated non-diabetic mice. The weight and volume of the SDF1α-treated fat grafts in the diabetic mice were significantly higher than the EPC-treated fat grafts in these mice (Figures 2B and 2D). The weight and volume of both SDF1α/AMD3100-treated fat grafts and SDF1α/L-NAME-treated fat grafts in diabetic mice were very small and significantly lower than the weight and volume of fat grafts found in the other groups (Figures 2B and 2D).

Flow cytometric analysis

To assess EPC kinetics, inventors performed a culture assay of peripheral blood (PB)- and bone marrow (BM)-MNCs by double staining for DiI-acLDL incorporation and BS-1 lectin binding. Representative images of cultured EPCs from PB-MNCs are shown in Figures 4A-Q. A greater number of EPCs in the BM was observed at day 18 in the SDF1α- and SDF1α/AMD3100-treated diabetic mice and a lower number of EPCs in the BM was observed at day 18 in the SDF1α/L-NAME-treated diabetic mice compared to those in the PBS-treated non-diabetic or PBS-treated diabetic mice (3.4-fold and 1.7-fold increase in the SDF1α group and SDF1α/AMD3100 group, respectively, and 2-fold decrease in the SDF1α/L-NAME group versus 1.1-fold increase in the PBS-treated non-diabetic mice and 1.2-fold decrease in the PBS-treated diabetic mice; n=4 for each group, $P<0.001$ for all measurements; Figure 3A). In the PB, the number of EPCs increased at day 18 in the SDF1α- and SDF1α/AMD3100-treated diabetic mice and decreased in the SDF1α/L-NAME-treated diabetic mice compared to

the PBS-treated non-diabetic or PBS-treated diabetic mice (3.3-fold and 3.2-fold increase in the SDF1α-treated and SDF1α/AMD3100-treated fat grafts in diabetic mice, respectively, and 2-fold decrease in the SDF1α/L-NAME-treated fat grafts in diabetic mice versus an unchanged number in the PBS-treated fat grafts in non-diabetic mice and 1.3 decrease in the PBS-treated diabetic fat grafts in diabetic mice; n=4 for each group, Figure 3B). These findings were confirmed with the data of FACS analysis of BM and PB samples collected at the same time points, indicating that the number of Sca-1^+/Flk-1^+ cells was consistently greater in the SDF1α- and SDF1α/AMD3100-treated fat grafts and lower in the L-NAME-treated fat grafts in diabetic mice than in the PBS-treated fat grafts in non-diabetic or diabetic mice (Figures 3C-D).

Augmentation of fat integration and neovascularization by SDF1α

Inventors examined whether EPC implantation with fat transplantation or treatment of fat grafts with SDF1α in diabetic mice could augment angiogenesis and fat integration in the fat grafts 15 weeks after fat transplantation. Figures 6A-D are photographs showing representative micrographs of fat integration and Figures 6E-H are photographs showing representative microvascular density (MVD) micrographs. The MVD in the SDF1α-treated fat grafts in the non-diabetic mice was significantly greater than that found in the PBS-treated fat grafts in non-diabetic mice ($P<0.001$; Figure 5A). The MVD in the EPC-treated fat grafts in the diabetic mice was not significantly different than that found in the PBS-treated fat grafts in the non-diabetic mice (P=NS). However the MVD in the SDF1α-treated fat grafts in the diabetic mice was significantly greater than that found in the EPC-treated fat grafts in the diabetic mice ($P<0.05$; Figure 5B) and significantly greater than that found in the SDF1α/AMD3100- or SDF1α/L-NAME-treated fat grafts in diabetic mice ($P<0.001$ for both; Figure 5B).

The findings of the histological analysis are summarized in Table 2, below, and representative findings are shown in Figures 6A-H. Integration, fibrosis, cyst and inflammation in the EPC-treated fat grafts in the diabetic mice were not different from the PBS-treated non-diabetic mice (Figures 6C and 6A, respectively). Nevertheless, inventors observed significantly higher integration and less inflammation in the SDF1α-treated fat grafts in the diabetic mice compared to those treated with EPCs in the diabetic mice (Figures 6D and 6C, respectively) or those treated with PBS in the non-diabetic mice (Figure 6A). Moreover, the SDF1α-treated fat grafts in either diabetic or non-

diabetic mice (Figures 6H and 6F, respectively) had well-vascularized areas with increased expression of CD31, and numerous endothelial islets in the granulation tissue compared to those treated with EPCs in diabetic mice (Figure 6G), those treated with PBS in non-diabetic mice (Figure 6E), and those treated with SDF1α/AMD3100 and SDF1α/L-NAME in diabetic mice (data not shown), all which were characterized with avascular areas, ectasic vessels with edema and perivascular hemorrhage, and a marked reduction in capillary ramification.

Table 2: Histological analysis

N	**Non Diabetic Control** *N=8*	**Non Diabetic SDF1α** *N=8*	**Diabetic EPC** *N=6*	**Diabetic SDF1α** *N=7*
Integration	3.30±1.00	**4.70±0.40***	3.20±1.20	**4.10±0.60****
Fibrosis	2.50±0.90	**0.90±0.20***	1.70±0.60	1.40±0.50
Cyst/Vacuoles	2.80±0.90	**1.00±0.20***	2.60±0.90	1.90±0.60
Inflammation	2.90±1.10	**0.70±0.10****	2.20±0.90	**1.10±0.40****

Values are presented as mean ± SD. (N) Number of mice.
Statistical significance is set at P<0.05. *P<0.05 **P<0.001 indicate difference from non diabetic mice whose fat grafts were treated with PBS.

Treatment with SDF1α augmented expression of angiogenic factors and reduced expression of apoptotic factors

Inventors investigated whether treatment of fat grafts with SDF1α upregulates the protein expression of VEGF, CXCR4, AKT and eNOS and downregulates the protein expression of caspase 3 and cytochrome c in the fat grafts at day 18 after fat transplantation. At post fat transplantation day 18, VEGF, CXCR4, AKT and eNOS protein expression increased significantly (2.8-fold, 4.2-fold, 3.1-fold and 3.7-fold respectively, n=4 for each group, P<0.001 for all measurements) in the EPC-treated fat grafts in diabetic mice compared to those treated with PBS in the diabetic mice. Treatment of the fat grafts with SDF1α in the diabetic mice increased VEGF, CXCR4, AKT and eNOS protein expression by 12.4-fold, 9.2-fold, 6.3-fold and 10.5-fold respectively (P<0.001 for all measurements). Treatment with AMD3100 of the diabetic

mice whose fat grafts were treated with SDF1α did not reduce the SDF1α-induced elevated VEGF and CXCR4 protein expression compared to that found in the SDF1α-treated fat grafts in the diabetic mice (P=NS for both) but abolished the protein expression of AKT and eNOS which were not different than those found in the PBS-treated fat grafts in the diabetic mice. Treatment with L-NAME of the diabetic mice whose fat grafts were treated with SDF1α abolished the SDF1α-induced elevated VEGF, CXCR4, AKT and eNOS protein expression and was not different than that found in the PBS-treated fat grafts of the diabetic mice (Figure 7A).

At day 18 post fat transplantation, caspase 3 and cytochrome c protein expression decreased significantly (3.8-fold and 5.2-fold, respectively, n=4 for each group, P<0.001 for both) in the EPC-treated fat grafts compared to the PBS-treated fat grafts in the diabetic mice. Treatment of the fat grafts with SDF1α decreased caspase 3 and cytochrome c protein expression by 6.8-fold and 15.3-fold, respectively (P<0.001 for both). Although, the expression levels of caspase 3 and cytochrome c proteins in the fat grafts of the AMD3100- or L-NAME-treated diabetic mice whose fat grafts were treated with SDF1α decreased significantly compared to those found in the fat grafts of the PBS-treated diabetic mice, they were still significantly greater than those found in the SDF1α-treated fat grafts of the diabetic mice (Figure 7B).

SDF1α augments in-vivo EPC migration and in-vitro tube formation

As illustrated in Figure 8A, EPC migration in diabetic mice was significantly lower than that found in the non-diabetic mice (P<0,001). Treatment of the fat grafts of diabetic mice with SDF1α increased EPC migration significantly at day 18 after fat transplantation compared to all the other groups (P<0.001). This increase in EPC migration returned to the same level of that found in the diabetic mice at week 15 after fat transplantation (P=NS). Intraperitoneal injection of AMD3100, L-NAME or anti-SDF1α neutralizing antibody significantly suppressed SDF1α-induced elevated EPC migration in the diabetic mice. Treatment of the fat grafts with EPCs had no effect on EPC migration in the diabetic mice.

As illustrated in Figure 8B, high glucose significantly decreased EPC tube formation compared to non-treated control EPCs (P<0.001). Treatment of the glucose stressed-EPCs by SDF1α resulted in a significant increase in EPC tube formation above that found in the glucose-stressed and non-stressed control EPCs (P<0.001 and P<0.05

respectively). Treatment of the glucose-stressed EPCs that were treated with SDF1α by AMD3100, L-NAME and anti-SDF1α neutralizing antibody significantly suppressed SDF1α-induced elevated EPC tube formation on matrigel.

In conclusion, the present inventors have shown that diabetes results in full fat resorption after fat transplantation, however, treatment of fat grafts with SDF1α maintains fat weight and volume in diabetic mice and results in higher fat weight and volume compared to those in non-diabetic mice. As shown above, possible mechanisms that underlie fat weight and volume maintenance by SDF1α in diabetic mice might be due to BM-EPC recruitment to the fat grafts that induce neovascularization and reduced fat cell apoptosis. Thus, SDF1α treatment of fat grafts promotes vasculogenesis and improves fat cell survival.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. A method of enhancing transplantation of a cell, a tissue or an organ graft in a subject in need thereof, the method comprising administering to a subject who has undergone transplantation of a cell, a tissue or an organ graft a therapeutically effective amount of SDF-1alpha, thereby enhancing transplantation of said cell, said tissue or said organ graft in the subject.

2. A method of enhancing survival of a cell, a tissue or an organ graft in a subject in need thereof, the method comprising:

(a) implanting a cell, a tissue or an organ graft into the subject; and

(b) administering to the subject a therapeutically effective amount of SDF-1alpha, thereby enhancing survival of said cell, said tissue or said organ graft in the subject.

3. A method of enhancing survival of a cell, a tissue or an organ graft in a subject in need thereof, the method comprising:

(a) contacting a cell, a tissue or an organ graft with SDF-1alpha; and

(b) implanting said cell, said tissue or said organ graft into the subject, thereby enhancing survival said cell, said tissue or said organ graft in the subject.

4. Use of SDF-1alpha for enhancing survival of a cell, a tissue or an organ graft in a subject in need thereof.

5. The method of claims 1, 2 or 3, or use of claim 4, wherein said cell or said tissue is a fat cell or fat tissue.

6. The method of claims 1, 2 or 3, or use of claim 4, wherein said cell or said tissue is a beta cell graft.

7. The method or use of claims 5 or 6, wherein the subject has diabetes.

8. The method or use of claim 5, wherein said fat cell or fat tissue is for treating a soft tissue defect.

9. The method or use of claim 8, wherein said soft tissue defect is selected from the group consisting of a skin condition, a skin malady, a wound, a burn, a cancer, a surgery, a reconstruction surgery, a skin depression, a congenital malformation and an acquired disease.

10. The method of claim 2, further comprising contacting said cell, said tissue or said organ graft with SDF-1alpha prior to said implanting.

11. The method of claim 2, wherein said subject is treated with a therapeutically effective amount of SDF-1alpha prior to said implanting of said cell, said tissue or said organ graft.

12. The method of claim 3, further comprising administering a therapeutically effective amount of SDF-1alpha to said subject following said implanting.

13. The method of claims 1, 2 or 3, wherein said administering is effected by direct injection of said SDF-1alpha into said cell, said tissue or said organ graft.

14. The method of claim 13, wherein a dose of said SDF-1alpha is about 1 pg/1,000,000 cells to about 1 mg/1,000,000 cells per injection.

15. The method of claims 1, 2 or 3, wherein said administering said SDF-1alpha is effected by a systemic route.

16. The method of claims 13 or 15, wherein a dose of said SDF-1alpha is about 1 pg/kg to about 1 mg/kg body weight.

17. The method of claims 1, 2, 11 or 12, wherein said therapeutically effective amount is for promoting angiogenesis and reducing apoptosis of said cell, said tissue or said organ graft.

18. The method of claims 1, 2 or 3, or use of claim 4, wherein the subject is diabetic.

19. The method of claims 1, 2 or 3, or use of claim 4, wherein the subject is a human subject.

20. The method of claim 1, 2 or 3, or use of claim 4, wherein said cell, said tissue or said organ graft is selected from the group consisting of a cardiac, a retina, a liver, a kidney, a lung, a splenic, a bone, a cartilage and a lymph cell, tissue or organ.

21. The method of claim 1, 2 or 3, or use of claim 4, wherein said tissue or said organ graft is a solid tissue or a solid organ.

22. The method of claim 1, 2 or 3, or use of claim 4, wherein said cell, said tissue or said organ graft is from an autologous origin.

23. The method of claim 1, 2 or 3, further comprising administering to the subject endothelial precursor cells (EPCs).

24. The method of claim 1, 2 or 3, further comprising administering to the subject at least one factor selected from the group consisting of an extracellular matrix component, a growth factor, a hormone, an angiogenic factor, a coagulation factor, a cytokine, a chemokine, an enzyme, a neurotransmitter, a vitamin, a carbohydrate, an ion, an iron chelator, a fatty acid, an antibiotic and an amino acid.

25. A pharmaceutical composition comprising a cell, a tissue or an organ graft, SDF-1alpha and a pharmaceutically acceptable carrier or diluent.

26. The pharmaceutical composition of claim 25, further comprising at least one factor selected from the group consisting of an extracellular matrix component, a growth factor, a hormone, an angiogenic factor, a coagulation factor, a cytokine, a chemokine, an enzyme, a neurotransmitter, a vitamin, a carbohydrate, an ion, an iron chelator, a fatty acid, an antibiotic, and an amino acid.

27. The pharmaceutical composition of claim 25, wherein a dose of said SDF-1alpha is about 1 pg/kg to about 1 mg/kg body weight.

28. A method of enhancing survival of a fat cell or fat tissue in a subject in need thereof, the method comprising:

(a) implanting a fat cell or fat tissue into the subject; and

(b) administering to the subject a therapeutically effective amount of SDF-1alpha, thereby enhancing survival of said fat cell or fat tissue.

29. The method of claim 28, further comprising contacting fat cell or fat tissue with SDF-1alpha prior to said implanting.

30. The method of claim 28, wherein said administering is effected systemically.

31. The method of claim 28, wherein said administering is effected locally.

32. The method of claim 28, wherein said fat cell or fat tissue is for treating a soft tissue defect.

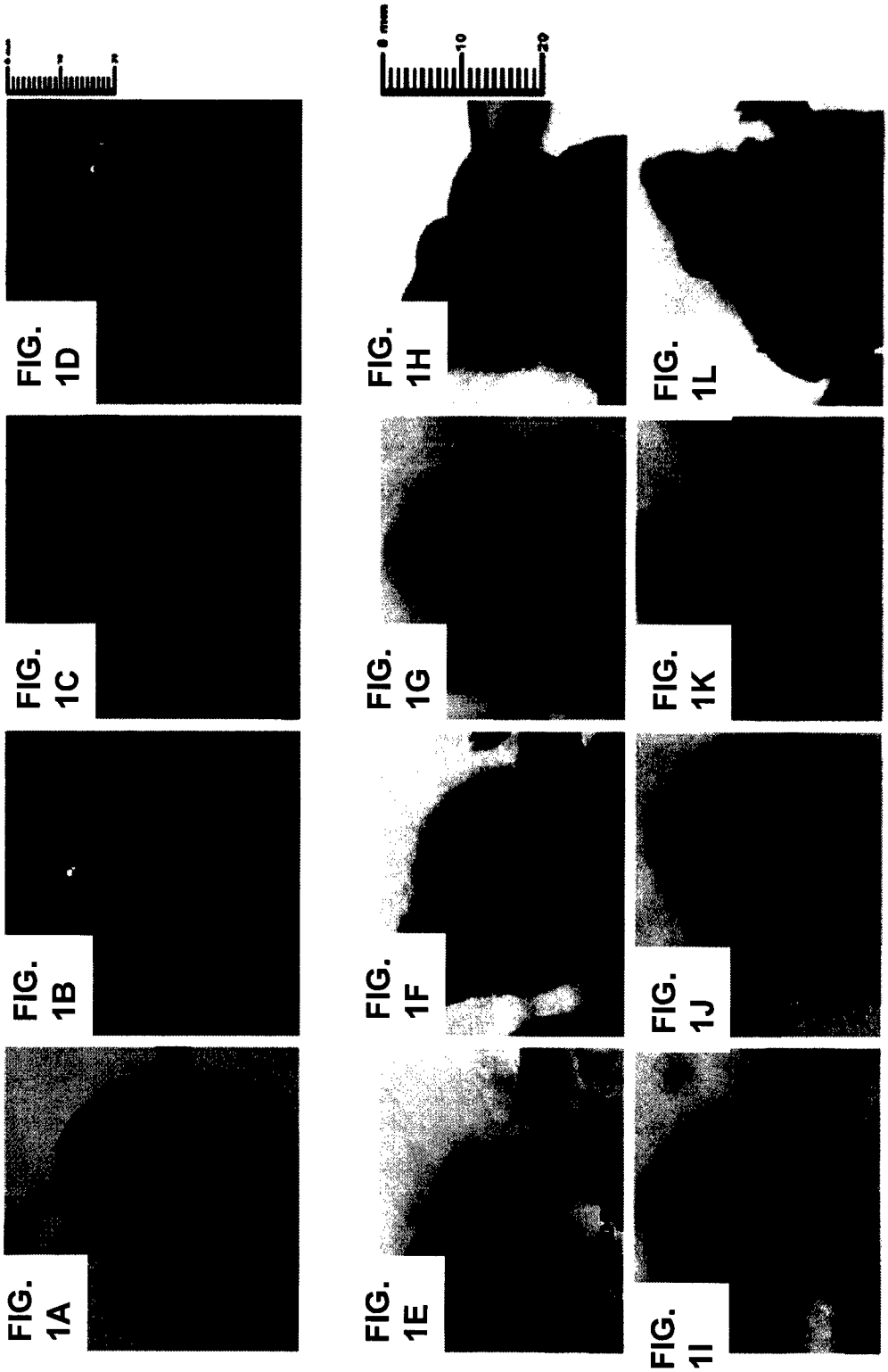


FIG. 1A
FIG. 1B
FIG. 1C
FIG. 1D
FIG. 1E
FIG. 1F
FIG. 1G
FIG. 1H
FIG. 1I
FIG. 1J
FIG. 1K
FIG. 1L

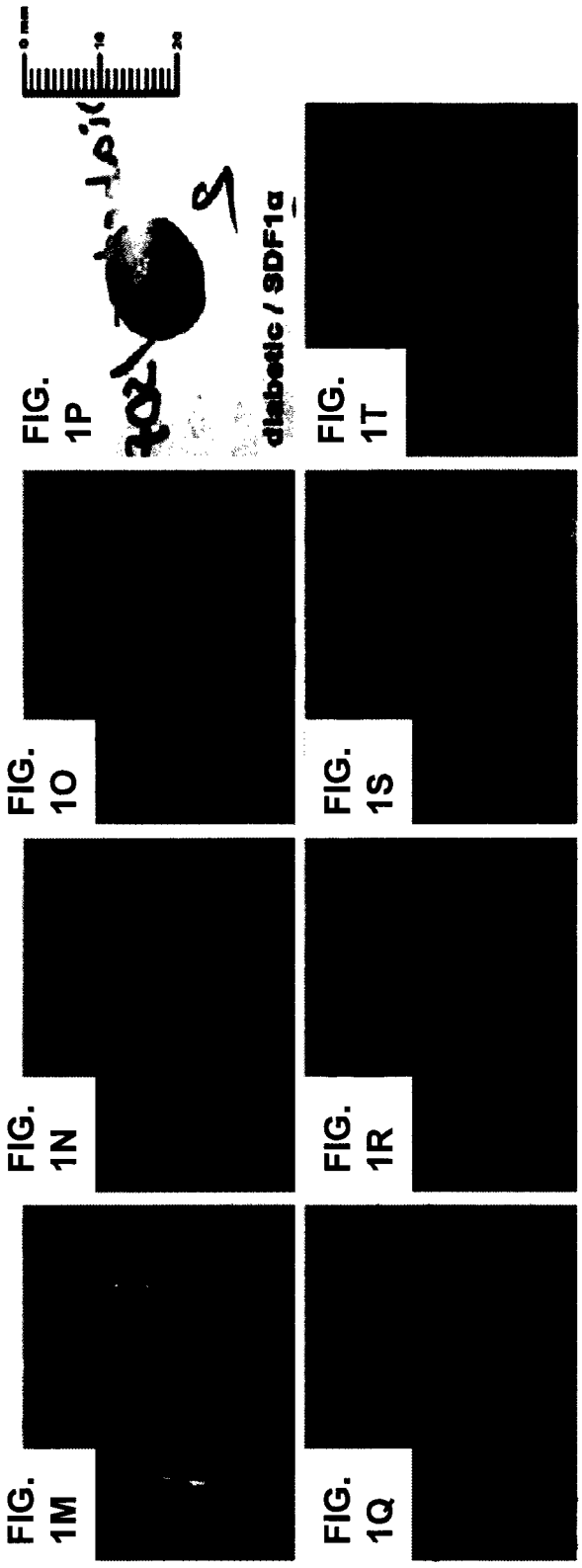


FIG. 1M
FIG. 1N
FIG. 1O
FIG. 1P
diabetic / SDF1α
FIG. 1Q
FIG. 1R
FIG. 1S
FIG. 1T

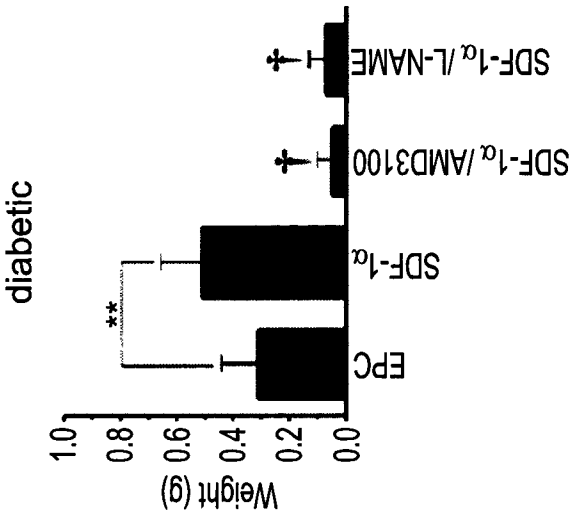


FIG. 2B

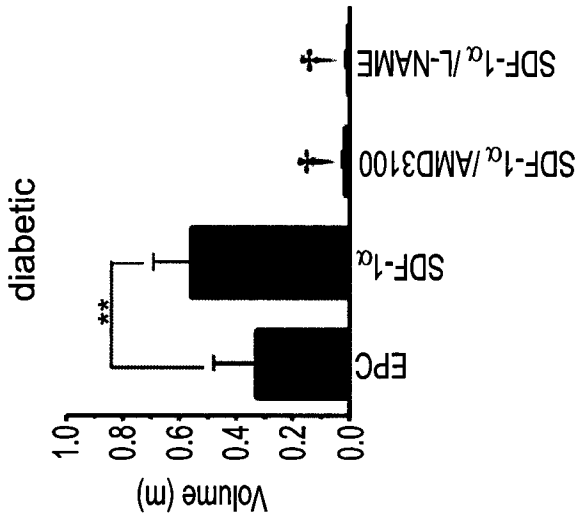


FIG. 2D

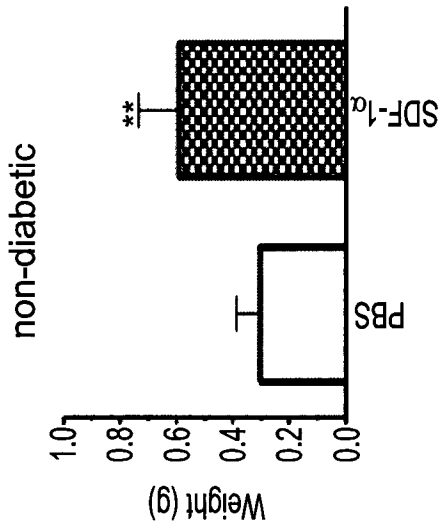


FIG. 2A

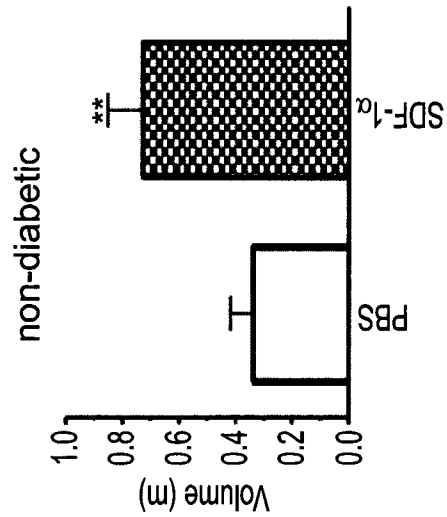


FIG. 2C

FIG. 3A BM-MNCs

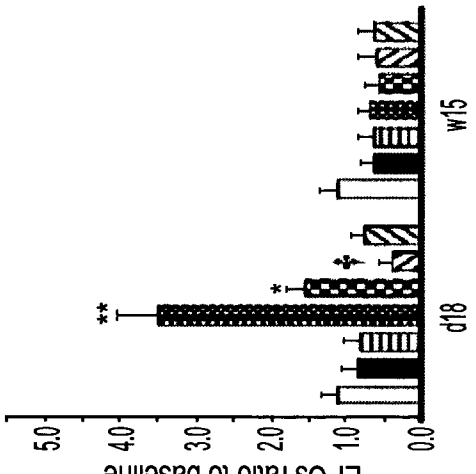


FIG. 3B PB-MNCs

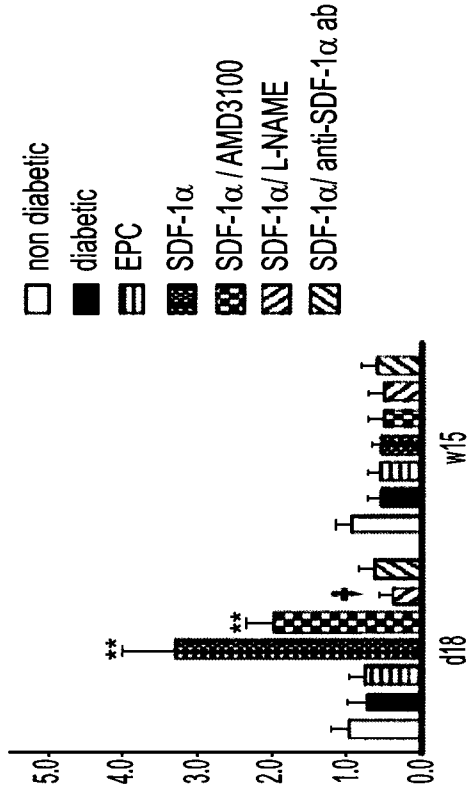


FIG. 3C BM-MNCs

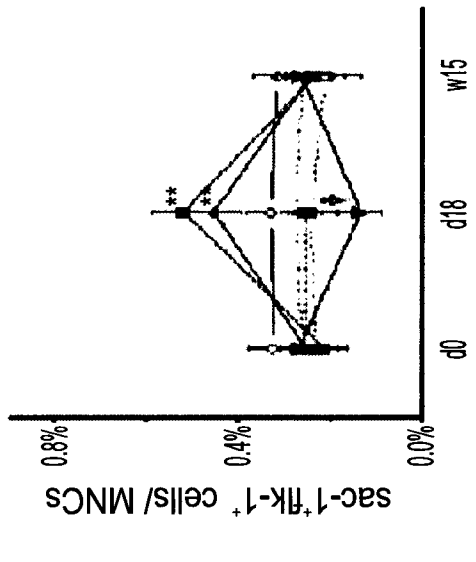
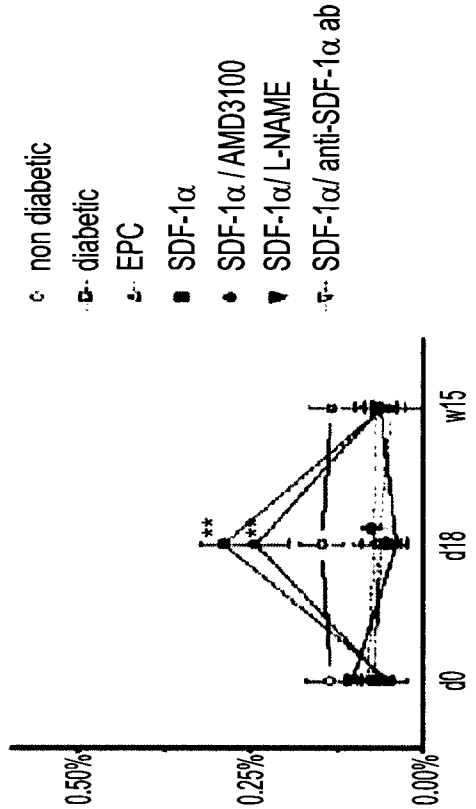


FIG. 3D PB-MNCs



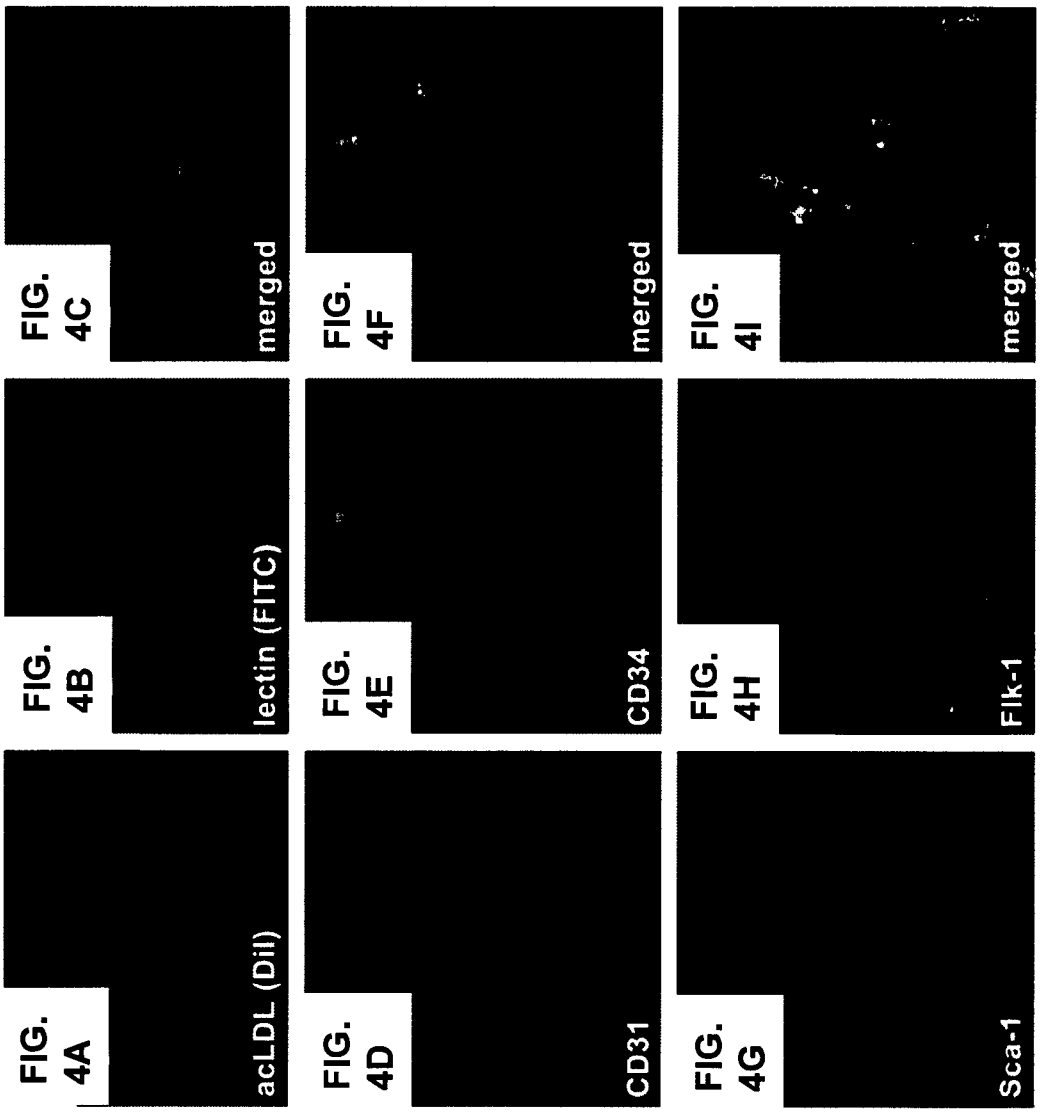


FIG. 4A
acLDL (DiI)
FIG. 4B
lectin (FITC)
FIG. 4C
merged
FIG. 4D
CD31
FIG. 4E
CD34
FIG. 4F
merged
FIG. 4G
Sca-1
FIG. 4H
Flk-1
FIG. 4I
merged

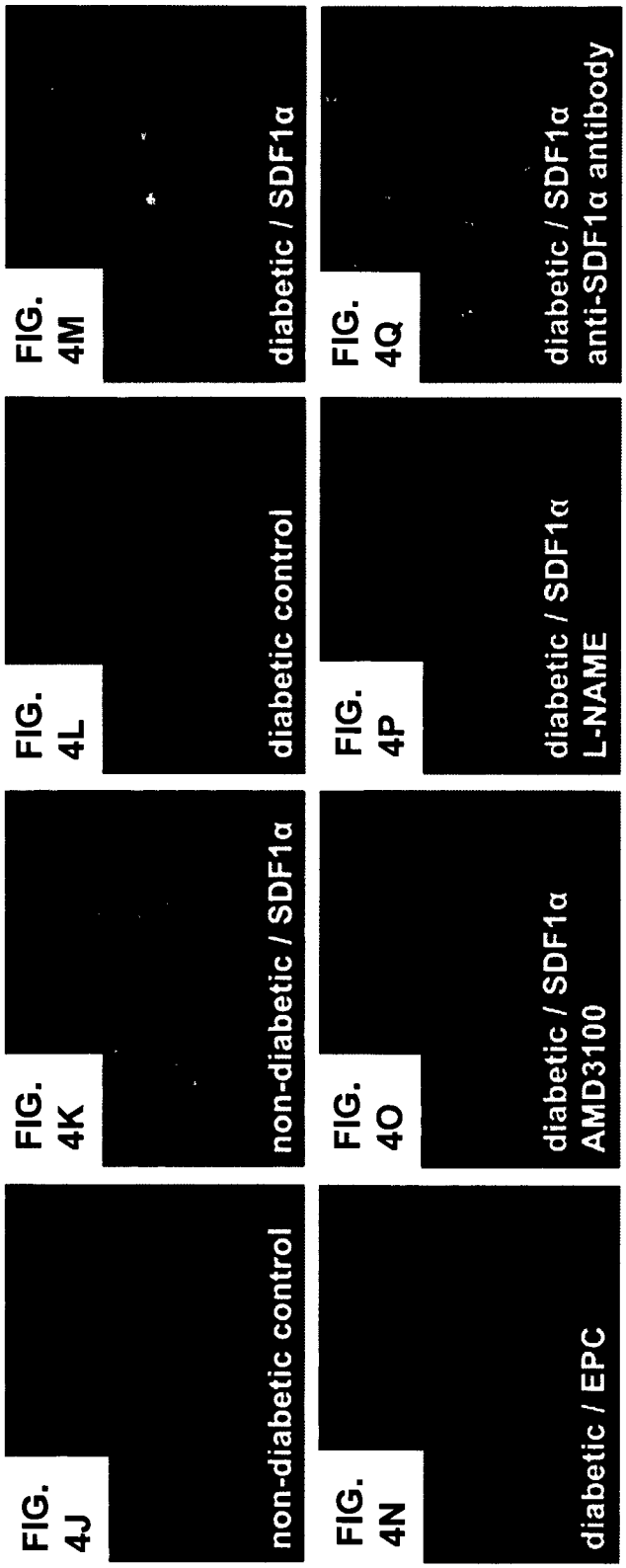


FIG. 4J
non-diabetic control
FIG. 4K
non-diabetic / SDF1α
FIG. 4L
diabetic control
FIG. 4M
diabetic / SDF1α
FIG. 4N
diabetic / EPC
FIG. 4O
diabetic / SDF1α AMD3100
FIG. 4P
diabetic / SDF1α L-NAME
FIG. 4Q
diabetic / SDF1α anti-SDF1α antibody

FIG. 5B

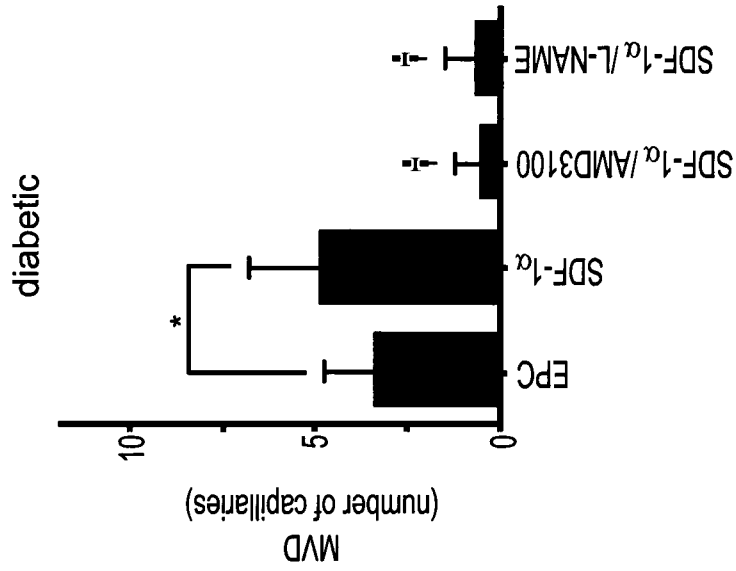
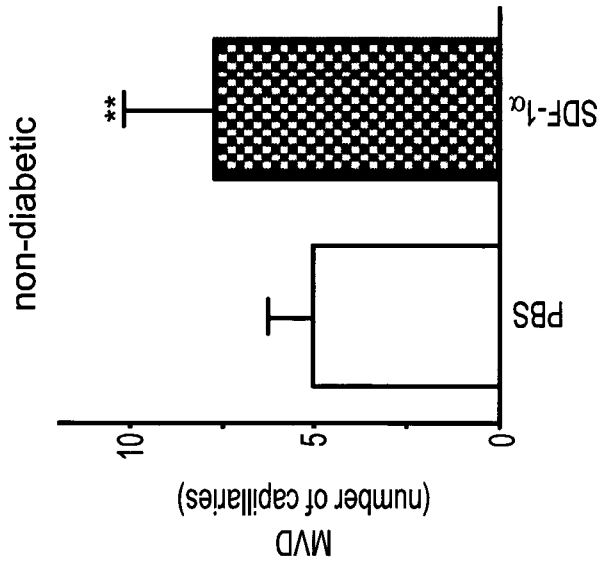

diabetic
MVD
(number of capillaries)
0
5
10
*
EPC
SDF-1α
SDF-1α /AMD3100
SDF-1α /L-NAME

FIG. 5A


non-diabetic
MVD
(number of capillaries)
0
5
10
**
PBS
SDF-1α

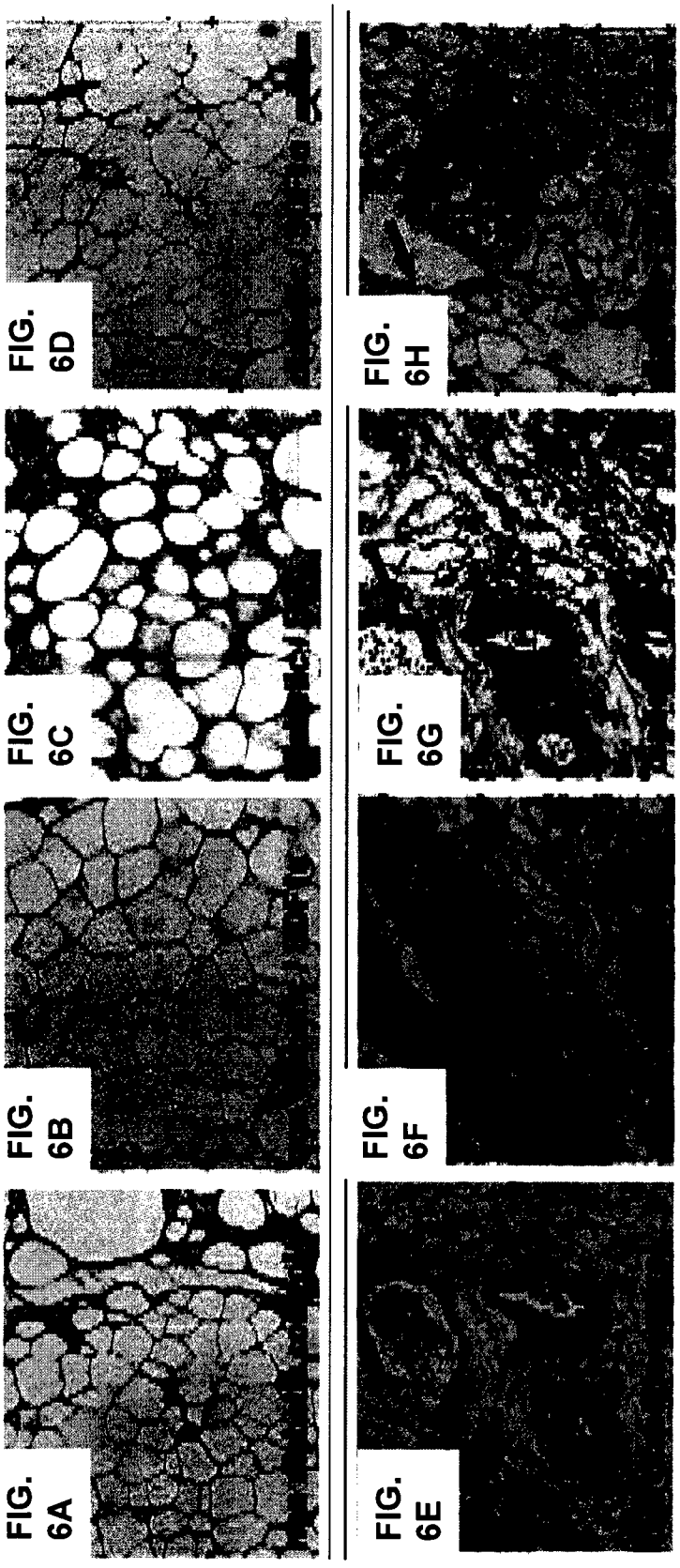


FIG. 6A
FIG. 6B
FIG. 6C
FIG. 6D
FIG. 6E
FIG. 6F
FIG. 6G
FIG. 6H

FIG. 7A

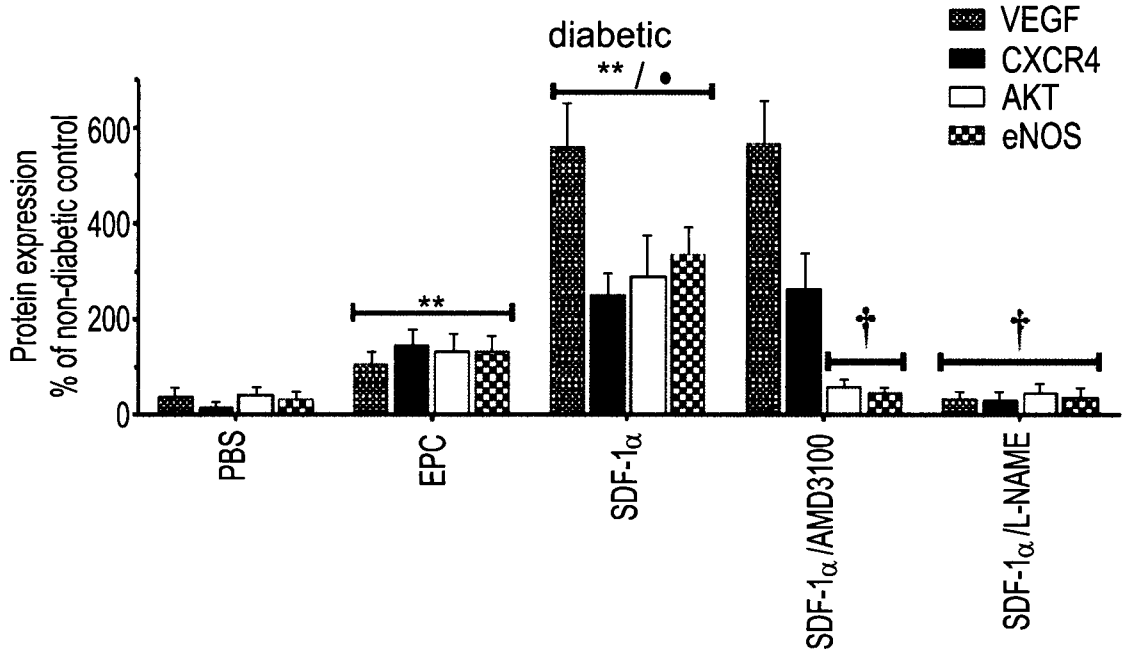

diabetic
VEGF
CXCR4
AKT
eNOS
Protein expression
% of non-diabetic control
600
400
200
0
**
** / •
†
†
PBS
EPC
SDF-1α
SDF-1α /AMD3100
SDF-1α /L-NAME

FIG. 7B

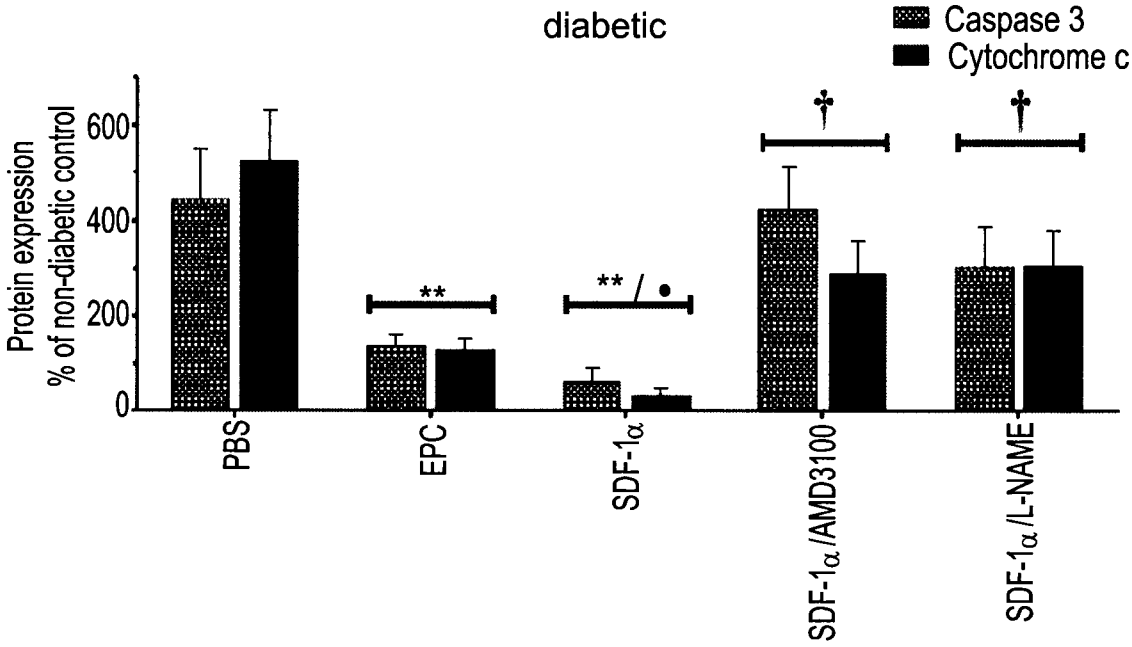

diabetic
Caspase 3
Cytochrome c
Protein expression
% of non-diabetic control
600
400
200
0
**
** / •
†
†
PBS
EPC
SDF-1α
SDF-1α /AMD3100
SDF-1α /L-NAME

FIG. 8A

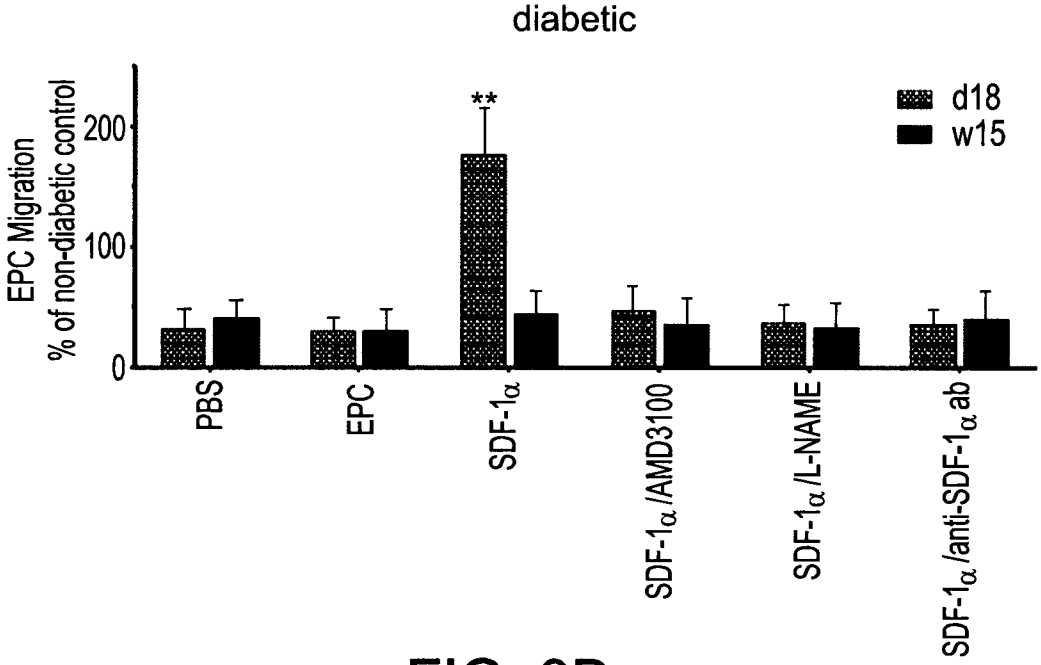
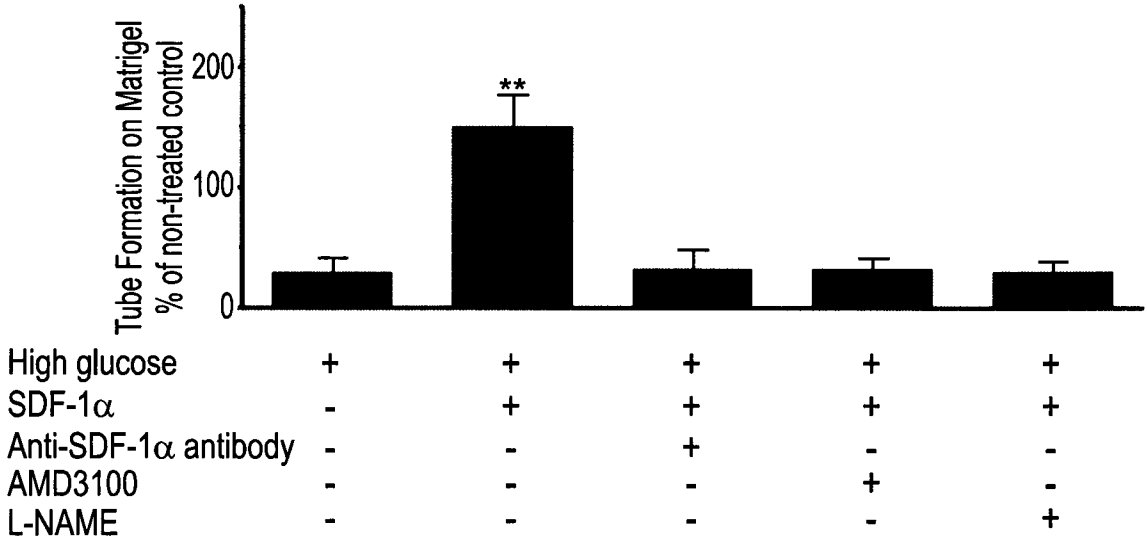

diabetic
EPC Migration
% of non-diabetic control
200
100
0
**
d18
w15
PBS
EPC
SDF-1α
SDF-1α /AMD3100
SDF-1α /L-NAME
SDF-1α /anti-SDF-1α ab

FIG. 8B


Tube Formation on Matrigel
% of non-treated control
200
100
0
**
High glucose + + + + +
SDF-1α - + + + +
Anti-SDF-1α antibody - - + - -
AMD3100 - - - + -
L-NAME - - - - +

INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2011/000683

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K35/00 A61K38/19 C12N5/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C12N

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/096359 A2 (RAPPAPORT FAMILY INST FOR RES [IL]; KARIN NATHAN [IL]; WILDBAUM GIZI [) 14 August 2008 (2008-08-14) page 4, lines 18-21 page 4, lines 22-26claim 2 -----	1,3,4,17
X	WO 2008/121719 A1 (CLEVELAND CLINIC FOUNDATION [US]; PENN MARC S [US]) 9 October 2008 (2008-10-09) paragraphs [0006], [0008] - [0009], [0039], [0020], [0056], [0146], [0147]; claims 50-52 -----	1-32
X	EP 1 726 311 A2 (YEDA RES AND DEV CO LTD AN ISR [IL] YEDA RES & DEV [IL]) 29 November 2006 (2006-11-29) paragraphs [0006], [0026], [0086], [0043]; example 2 ----- -/--	1-32

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 January 2012	30/01/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Habedanck, Robert

INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2011/000683

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WO 2006/032075 A1 (ANGIOBLAST SYSTEMS INC [US]; GRONTHOS STAN [AU]; ZANNETTINO ANDREW CHR) 30 March 2006 (2006-03-30) page 4, lines 7-16 page 14, lines 3-10 page 17, lines 20-36 page 2, lines 14-18 page 23, lines 4-25 page 20, lines 21-35 page 4, lines 17-25 page 4, lines 27-33 page 15, lines 31-35examples 9-10	1-32
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X	H. E. BROXMEYER: "Stromal cell-derived factor-1/CXCL12 directly enhances survival/antiapoptosis of myeloid progenitor cells through CXCR4 and Galphai proteins and enhances engraftment of competitive, repopulating stem cells", JOURNAL OF LEUKOCYTE BIOLOGY, vol. 73, no. 5, 1 May 2003 (2003-05-01), pages 630-638, XP055015092, ISSN: 0741-5400, DOI: 10.1189/jlb.1002495 abstract page 631, left column, paragraph 2 page 631, right column, paragraph 1 page 635, left column, line 11-page 636, left column, line 2figures 1-2,6-7	1-32
X,P	WANG N ET AL: "Endothelial progenitor cells pre-cultured with stromal cell derived factor 1 alpha transplantation to treat hind limb ischemia in diabetic rats", ZHONGGUO ZUZHI GONGCHENG YU LINCHUANG KANGFU= JOURNAL OF CLINICAL REHABILITATIVE TISSUE ENGINEERING RESEARCH, ZHONGGUO KANGFU YIXUEHUI, CN, vol. 15, no. 36, 1 January 2011 (2011-01-01), pages 6749-6752, XP009154970, ISSN: 1673-8225 abstract	1-23

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2011/000683

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JIAN-XING YU ET AL: "Combination of stromal-derived factor-1[alpha] and vascular endothelial growth factor gene-modified endothelial progenitor cells is more effective for ischemic neovascularization", JOURNAL OF VASCULAR SURGERY, vol. 50, no. 3, 1 September 2009 (2009-09-01), pages 608-616, XP055014974, ISSN: 0741-5214, DOI: 10.1016/j.jvs.2009.05.049 abstractfigures 2-4	1-32
X,P	DI ROCCO GIULIANA ET AL: "Enhanced healing of diabetic wounds by topical administration of adipose tissue-derived stromal cells overexpressing stromal-derived factor-1: biodistribution and engraftment analysis by bioluminescent imaging.", STEM CELLS INTERNATIONAL, vol. 2011, 304562, 2010, pages 1-11, XP002666787, ISSN: 1687-9678 abstract	1-32
A	WO 00/06704 A2 (YEDA RES & DEV [IL]; LAPIDOT TSVEE [IL]; PELED AMNON [IL]) 10 February 2000 (2000-02-10)	1-32
A	YAMAGUCHI J-I ET AL: "Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization", CIRCULATION, vol. 107, no. 9, 11 March 2003 (2003-03-11), pages 1322-1328, XP002305134, LIPPINCOTT WILLIAMS & WILKINS, US ISSN: 0009-7322, DOI: 10.1161/01.CIR.0000055313.77510.22 abstract	1-32

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2011/000683

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THEVENOT P T ET AL: "The effect of incorporation of SDF-1@? into PLGA scaffolds on stem cell recruitment and the inflammatory response", BIOMATERIALS, vol. 31, no. 14, 1 May 2010 (2010-05-01), pages 3997-4008, XP026947574, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB ISSN: 0142-9612, DOI: 10.1016/J.BIOMATERIALS.2010.01.144 [retrieved on 2010-02-24] abstract -----	1-32
A	ELMADBOUH I ET AL: "Ex vivo delivered stromal cell-derived factor-1alpha promotes stem cell homing and induces angiomyogenesis in the infarcted myocardium", JOURNAL OF MOLECULAR AND CELLULAR CARDIOLOGY, vol. 42, no. 4, 1 April 2007 (2007-04-01), pages 792-803, XP002643111, ACADEMIC PRESS, GB ISSN: 0022-2828, DOI: 10.1016/J.YJMCC.2007.02.001 [retrieved on 2007-02-08] abstract -----	1-32

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IL2011/000683

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