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(54) Title: STEM CELL THERAPY FOR WEIGHT LOSS

(57) Abstract: Disclosed are methods, cells, and compositions of matter useful for the treatment of obesity, including but not limited to, diminishment of rate of weight gain, maintenance of body weight, or induction of weight loss. The invention teaches particular administration of various cell populations, including mononuclear cells from adipose tissue, in order to directly induce weight loss or activate biological pathways whose effects culminate in weight loss. The invention may also be utilized within the context of existing weight loss programs in order to augment their efficacy.

STEM CELL THERAPY FOR WEIGHT LOSS

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No: 60/977,607 filed on October 4, 2007, the contents of which are hereby incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] The invention pertains to the field of cellular therapy, particularly, in some aspects, the invention pertains to the field of metabolic manipulation by administration of cells. The invention relates to the induction of weight loss through the systemic administration of various stem cell types including autologous, allogeneic or xenogeneic.

Description of the Related Art

[0003] Several methods of defining obesity exist, the most common one being based on the body mass index (BMI), a figure calculated based on the person's weight and height. Generally, when the BMI is between 18.5 to 25 the person is considered healthy. BMI of 25-30 is considered overweight, 30-40 is considered obese, and 40 and over is considered morbidly or severely obese. Obesity is considered an epidemic in the United States and Western Europe. In fact, more than half of Americans are overweight. Nearly one third are considered obese. The detrimental effects of obesity oN overall health can be seen by the fact that obesity is the 2nd largest preventable killer of Americans, the first being tobacco use. It is estimated that every year billions of dollars are spent on dieting, diet foods, diet books, diet pills, and the like in the US. Another $45 billion is spent on treating the diseases associated with obesity. Furthermore, businesses suffer an estimated $20 billion loss in productivity each year from absence due to illness caused by obesity.

[0004] Methods of currently treating obesity include nutritional modification, exercise, medications, and surgery. Numerous diets have been proposed, some with more

efficiency thaN others at evoking weight loss. In numerous cases patients find it difficult to observe various diets and in some cases this results in extremes between overindulging and overdieting. Medications for the treatment of obesity include Orlistat (Xenical), which is prescribed if the patient has a BMI of >30. Orlistat is an inhibitor of pancreatic lipase which prevents metabolism of triglycerides into free fatty acids. Since triglycerides are not readily absorbable, they are excreted, and as a result the caloric intake attributed to metabolized free fatty acids is reduced. Unfortunately, Orlistat is not associated with long-term weight reduction and in some patients causes numerous side effects. Phentermine is a centrally acting stimulant that is effective at reducing weight for limited periods of time. It is advisable not to utilize it for more than 12 weeks due to possibility of dependency as well as numerous adverse effects such as development of heart valve failure. Surgical approaches to obesity have included procedures such as laparoscopic banding, in which a device is used to "tie off" or constrict a portion of the stomach, or the placement of intragastric balloon in order to limit the amount of food entering the stomach. Endoscopic procedures have historically focused on the placement of a balloon or other space occupying device in the patient's stomach to fill portions of the stomach and provide the patient with the feeling of fullness, thereby reducing food intake.

[0005] Today there appears to be a profound need for novel interventions that address the problem of obesity without demanding severe changes in the lifestyle of the patient. Current approaches either require significant effort (exercise, dieting, etc) and therefore have low compliance, or are associated with highly invasive procedures (bariatric surgery, laparascopic banding, etc).

SUMMARY OF THE INVENTION

[0006] Presented herein are the unexpected findings that administration of mononuclear cells from adipose tissue results in the induction of weight loss. The current invention addresses the problem of obesity through activation of endogenous self-controlling pathways through the administration of regenerative cells or stem cells. Accordingly, given the various regenerative properties of mononuclear cells derived from adipose tissue, the invention teaches that such regenerative cells may be used for the treatment of obesity in general, but also for individuals in which weight loss is desired.

[0007] Accordingly, presented herein is a method of treating obesity in a subject comprising the steps of: a) selecting a patient in need of treatment for obesity; and b) providing or administering a therapeutically effective amount of purified mononuclear cells to the patient. In certain aspects, the mononuclear cells have been isolated from tissue of the patient.

[0008] Also provided herein is a method of treating obesity in a subject comprising providing a therapeutically effective amount of purified mononuclear cells to a patient in need of or selected for treatment for obesity.

[0009] Also presented herein is a method of preventing secondary complications associated with obesity comprising the steps of: a) selecting a patient in need of treatment for secondary complications associated with obesity; and b) providing or administering a therapeutically effective amount of purified mononuclear cells to the patient. In certain aspects, the mononuclear cells can be isolated from tissue of the patient.

[0010] Also provided herein is a method of preventing secondary complications associated with obesity in a subject comprising providing a therapeutically effective amount of purified mononuclear cells to a patient in need of or selected for treatment for secondary complications associated with obesity.

[0011] Also presented herein is a method of treating obesity in a subject comprising the steps of: a) withdrawing an amount of adipose tissue from the subject; b) substantially purifying mononuclear cells from the adipose tissue; and c) reintroducing a therapeutically effective amount of purified mononuclear cells to the subject.

[0012] In certain aspects, purified mononuclear cells can be substantially free of adipocytes.

[0013] In certain aspects, the purified mononuclear cells can be administered systemically.

[0014] In certain aspects, subsequent to isolation of the substantially purified mononuclear cells, the cells can be divided into aliquots, with some aliquots being frozen for future administration, while some aliquots are administered to the subject after purification.

[0015] In certain aspects, the number of purified mononuclear cells re-introduced into the patient can be adjusted based on extent of desired weight loss.

[0016] In certain aspects, the number of purified mononuclear cells reintroduced into the patient can be adjusted based on modification of metabolic parameters.

[0017] In certain aspects, the obesity can be associated with a metabolic dysfunction.

[0018] In certain aspects, metabolic dysfunction can be associated or caused by a condition selected from the group of the following conditions: Prader-Willi syndrome, Cushing syndrome, drug-induced metabolic dysfunction, congenital metabolic dysfunctions, hypothyroidism and growth hormone deficiency.

[0019] Also presented herein is a method of preventing secondary complications associated with obesity comprising the steps of: a) withdrawing an amount of adipose tissue from the subject; b) substantially purifying mononuclear cells from the adipose tissue; and c) reintroducing a therapeutically effective amount of purified mononuclear cells to the subject.

[0020] In certain aspects, purified mononuclear cells can be substantially free of adipocytes.

[0021] In certain aspects, the purified mononuclear cells can be administered systemically.

[0022] In certain aspects, subsequent to isolation of the substantially purified mononuclear cells, the cells can be divided into aliquots, with some aliquots being frozen for future administration, while some aliquots are administered to the subject after purification.

[0023] In certain aspects, the number and/or location of purified mononuclear cells re-introduced into the patient can be adjusted based on extent of desired protective and/or therapeutic effect on the secondary complication associated with obesity.

[0024] In certain aspects, the secondary complication of obesity can be selected from a group consisting of: atherosclerosis, endothelial dysfunction, coronary artery disease, insulin resistance, cor pulmonale, pulmonary hypertension, left ventricular hypertrophy, cardiomyopathy, dyslipidemia, obstructive sleep apnea, systemic hypertension, renal failure, stress incontinence, fatty liver disease, and predisposition to stroke.

[0025] Also presented herein is a method of treating obesity in a subject comprising the steps of: a) selecting a stem cell source; b) purifying and/or concentrating

stem cells from the stem cell source; and c) reintroducing a therapeutically effective amount of purified mononuclear cells to the subject.

[0026] In certain aspects, the stem cell source can be selected from a group consisting of: autologous, allogeneic, and xenogeneic.

[0027] In certain aspects, the stem cell source can be selected from a group consisting of: bone marrow, peripheral blood, mobilized peripheral blood, cord blood, menstrual blood, wharton's jelly, placental matrix, embryonic, fetal, retrodifferentiated tissue, side population cells, amnion, amniotic fluid, deciduous tooth, hair follicle, paraventricular zone, and adipose tissue.

[0028] In certain aspects, purified mononuclear cells can be extracted from the stem cell source and administered into a patient in need thereof as a heterogenous population containing stem cells and other mononuclear cells specific to the tissue.

[0029] In certain aspects, the purified mononuclear cells can be enriched for cells with stem cell activity.

[0030] In certain aspects, the cells with stem cell activity are enriched by a method selected from a group of methods consisting of: a) selection based on P-glycoprotein drug efflux pump activity as measured by extrusion of a substrate, the representative substrate being rhodamine 123; b) selection based on multidrug resistance protein-1 activity as measured by extrusion of a substrate, the representative substrate being Fluo-3; c) selection based on aldehyde dehydrogenase activity as measured by ALDEFLUOR staining; d) selection based on expression of CD117; e) selection based on expression of CD133; f) selection based on expression of CD34; and g) selection based on markers associated with high replicative potential cells.

[0031] In certain aspects, the purified mononuclear cells can be substantially free of non-stem cells.

[0032] In certain aspects, the purified mononuclear cells can be administered systemically.

[0033] In certain aspects, subsequent to isolation of the substantially purified mononuclear cells, the cells are divided into aliquots, with some aliquots being frozen for

future administration, while some aliquots can be administered to the subject after purification.

[0034] In certain aspects, the number and/or location of purified mononuclear cells re-introduced into the patient is adjusted based on extent of desired protective and/or therapeutic effect on the secondary complication associated with obesity.

[0035] Also presented herein is a method of preventing secondary complications associated with obesity comprising the steps of: a) selecting a stem cell source; b) purifying and/or concentrating stem cells from the stem cell source; and c) reintroducing a therapeutically effective amount of purified mononuclear cells to the subject.

[0036] In certain aspects, the stem cell source can be selected from a group consisting of: autologous, allogeneic, and xenogeneic.

[0037] In certain aspects, the stem cell source can be selected from a group consisting of: bone marrow, peripheral blood, mobilized peripheral blood, cord blood, menstrual blood, wharton's jelly, placental matrix, embryonic, fetal, retrodifferentiated tissue, side population cells, amnion, amniotic fluid, deciduous tooth, hair follicle, paraventricular zone, and adipose tissue.

[0038] In certain aspects, purified mononuclear cells can be extracted from the stem cell source and administered into a patient in need thereof as a heterogenous population containing stem cells and other mononuclear cells specific to the tissue.

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[0040] In certain aspects, the cells with stem cell activity can be enriched by a method selected from a group of methods consisting of: a) selection based on P-glycoprotein drug efflux pump activity as measured by extrusion of a substrate, the representative substrate being rhodamine 123; b) selection based on multidrug resistance protein-1 activity as measured by extrusion of a substrate, the representative substrate being Fluo-3; c) selection based on aldehyde dehydrogenase activity as measured by ALDEFLUOR staining; d) selection based on expression of CD117; e) selection based on expression of CD133; f) selection based on expression of CD34; and g) selection based on markers associated with high replicative potential cells.

[0041] In certain aspects, the purified mononuclear cells can be substantially free of non-stem cells.

[0042] In certain aspects, the purified mononuclear cells can be administered systemically.

[0043] In certain aspects, subsequent to isolation of the substantially purified mononuclear cells, the cells can be divided into aliquots, with some aliquots being frozen for future administration, while some aliquots are administered to the subject after purification.

[0044] In certain aspects, the number and/or location of purified mononuclear cells re-introduced into the patient can be adjusted based on extent of desired protective and/or therapeutic effect on the secondary complication associated with obesity.

[0045] In certain aspects, the secondary complication of obesity can be selected from a group consisting of: atherosclerosis, endothelial dysfunction, coronary artery disease, insulin resistance, cor pulmonale, pulmonary hypertension, left ventricular hypertrophy, cardiomyopathy, dyslipidemia, obstructive sleep apnea, systemic hypertension, renal failure, stress incontinence, fatty liver disease, and predisposition to stroke.

[0046] Also presented herein is a method of inducing weight loss in a subject through the administration of purified mononuclear cells as described above.

[0047] Also presented herein is a method of increasing efficacy of a therapy or intervention that induces weight loss through the administration of purified mononuclear cells as described above.

[0048] In any of the above embodiments where an agent capable of mobilizing endogenous stem cells is administered, a range of effective concentrations can be used. For example, where G-CSF is administered, G-CSF can be administered at a concentration ranging from about 0.01, about 0.1, about 1 to about 5000, about 2000, about 1000, about 900, about 800, about 700, about 600, about 500, about 400, about 300, about 200, about 100, about 50, about 25, about 10, 9, 8, 7, 6, 5, 4, 3, or about 2 micrograms/kilogram of patient body weight per day for a period ranging from about 1 day to about 100 days. Other similar dose ranges are applicable for agent such as: M-CSF, G-CSF, GM-CSF, an antagonist of CXCR-4, an antagonist of VLA-4, fucoidan, IVIG, parathyroid hormone, and cyclophosphamide.

[0049] In any of the above embodiments, the amount of purified mononuclear cells administered can be at least 1, at least 10, at least 100, at least 1,000, at least 10,000, at least 100,000, at least 1 million, at least 10 million, or at least 100 million cells. The amount of cells administered can range from 1 cell to about 100 million cells, from about 100 cells to about 10 million cells, from about 1000 cells to about 10 million cells, from about 10,000 cells to about 10 million cells, from about 100,000 cells to about 10 million cells, from about 1 million cells to about 10 million cells, and from about 1 million cells to about 5 million cells.

[0050] In one embodiment of the invention, mononuclear cells are concentrated in an injection solution, which may be saline, mixtures of autologous plasma together with saline, or various concentrations of albumin with saline. Typically the pH of the injection solution is from about 6.4 to about 8.3, optimally 7.4. Excipients may be used to bring the solution to isotonicity such as, 4.5% mannitol or 0.9% sodium chloride, pH buffers with art-known buffer solutions, such as sodium phosphate. Other pharmaceutically acceptable agents can also be used to bring the solution to isotonicity, including, but not limited to, dextrose, boric acid, sodium tartrate, propylene glycol, polyols (such as mannitol and sorbitol) or other inorganic or organic solutes.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0051] Presented herein are the unexpected findings that administration of mononuclear cells from adipose tissue results in the induction of weight loss. Certain embodiments described herein address the problem of obesity through activation of endogenous self-controlling pathways through the administration of regenerative cells or stem cells. Accordingly, given the various regenerative properties of mononuclear cells derived from adipose tissue, such regenerative cells may be used for the treatment of obesity in general, but also for individuals in which weight loss is desired.

[0052] As used herein, the term therapeutically effective amount refers to an amount or concentration which is effective in reducing, eliminating, treating, preventing or controlling the symptoms of a pervasive developmental disorder affecting a mammal. The term controlling is intended to refer to all processes wherein there may be a slowing,

interrupting, arresting or stopping of the progression of the pervasive developmental disorder affecting the mammal. However, controlling does not necessarily indicate a total elimination of all disease and condition symptoms, and is intended to include prophylactic treatment.

[0053] In one aspect, liposuction is performed on patients in whom weight-loss is desired. Although liposuction itself induces weight loss, this weight loss is transient. Indeed, within the context of the invention, the induction of weight loss is not mediated through the simple removal of adipose tissue, but through the biological cascades that are activated in the host subsequent to the readministration of mononuclear cells from the adipose tissue into systemic circulation. Accordingly, in one embodiment of the invention, mononuclear cells are derived from adipose tissue and administered to a patient in need thereof.

[0054] Mononuclear cells derived from adipose tissue may be obtained by any method known to those of skill in the art. For example, adipose tissue can be readily obtained as a result of liposuction procedures. The adipose tissue may be from the patient who is to be treated, or from another source. Where adipose tissue is obtained as a result of liposuction procedures, standard methods are known in the art for isolating mononuclear cells. Specifically, for example, liposuction can be performed so as to attain 10-200 ml of raw lipoaspirate. The lipoaspirate is washed in phosphate buffered saline and digested with 0.075% collagenase type I for 30-60 min at 37°C by gentle agitation. After the digestion is completed neutralization of the collagenase with DMEM or other medium containing autologous serum is performed, preferably at a concentration of 10% v/v autologous serum to media. The cells are centrifuged at approximately 700-2000g for 5-15 minutes, followed by resuspension of the cells in an appropriate medium such as DMEM. Cells are then filtered using a cell strainer, for example a 100 μm nylon cell strainer in order to remove debris. Filtered cells are subsequently centrifuged again at approximately 700-2000g for 5-15 minutes and resuspended at a concentration of approximately 1x106/cm2 into culture flasks or similar vessels. After 10-20 hours of culture non-adherent cells are removed by washing with PBS and remaining cells are cultured. Upon reaching a concentration desired for clinical use, cells are harvested, assessed for purity and administered in a patient in need thereof as described above. In some embodiments the invention is practiced without the need for culture and the adipose derived mononuclear cells are used as freshly isolated. In some

embodiments it may be desirous to increase the number of adipose derived stem cells in the adipose derived stem cells in the inoculum administered. For this, markers specific to stem cells found in adipose tissue may be used in order to obtain high stem cell yields.

[0055] Stem cells are known to express markers such as CD9; CD29 (integrin beta 1); CD44 (hyaluronate receptor); CD49d,e (integrin alpha 4, 5); CD55 (decay accelerating factor); CD105 (endoglin); CD106 (VCAM-1); CD166 (ALCAM). These markers are useful not only for identification but may be used as a means of positive selection, before and/or after culture in order to increase purity of the desired cell population. In terms of purification and isolation, devices are known to those skilled in the art for rapid extraction and purification of cells adipose tissues. U.S. Patent No. 6,316,247 (incorporated herein by reference in its entirety) describes a device which purifies mononuclear adipose derived stem cells in an enclosed environment without the need for setting up a GMP/GTP cell processing laboratory so that patients may be treated in a wide variety of settings.

Sources of Stem Cells

[0056] Also presented herein is a method of treating obesity in a subject comprising the steps of: a) selecting a stem cell source; b) purifying and/or concentrating stem cells from the stem cell source; and c) reintroducing a therapeutically effective amount of purified mononuclear cells to the subject. In certain aspects, the stem cell source can be selected from a group consisting of: autologous, allogeneic, and xenogeneic.

[0057] In certain aspects, the stem cell source can be selected from a group consisting of: bone marrow, peripheral blood, mobilized peripheral blood, cord blood, menstrual blood, wharton's jelly, placental matrix, embryonic, fetal, retrodifferentiated tissue, side population cells, amnion, amniotic fluid, deciduous tooth, hair follicle, paraventricular zone, and adipose tissue.

Cord blood stem cells

[0058] In certain embodiments of the invention cord blood stem cells are administered to a patient suffering from obesity. Said cord blood stem cells may be administered as a heterogenous population of cells by the administration of cord blood

mononuclear cells. Said cells may be isolated according to many methods known in the art. In one particular method, cord blood is collected from fresh placenta and mononuclear cells are purified by centrifugation using a density gradient such as Ficoll or Percoll, in another method cord blood mononuclear cells are isolated from contaminating erythrocytes and granulocytes by the Hetastarch with a 6% (wt/vol) hydroxyethyl starch gradient. Cells are subsequently washed to remove contaminating debris, assessed for viability, and administered at a concentration and frequency sufficient to induce therapeutic benefit.

[0059] In another embodiment of the invention, cord blood stem cells are fractionated and the fraction with enhanced therapeutic activity is administered to the patient. Enrichment of cells with therapeutic activity may be performed using physical differences, electrical potential differences, differences in uptake or excretion of certain compounds, as well as differences in expression marker proteins. Distinct physical property differences between stem cells with high proliferative potential and low proliferative potential are known. Accordingly, in some embodiments of the invention, it will be useful to select cord blood stem cells with a higher proliferative ability, whereas in other situations, a lower proliferative ability may be desired. In some embodiments of the invention, cells are directly injected into the area of need, such as in the corpora cavernosa, in which case it will be desirable for said stem cells to be substantially differentiated, whereas in other embodiments, cells will be administered systemically and it this case with may be desirable for the administered cells to be less differentiated, so has to still possess homing activity to the area of need. In embodiments of the invention where specific cellular physical properties are the basis of differentiating between cord blood stem cells with various biological activities, discrimination on the basis of physical properties can be performed using a Fluorescent Activated Cell Sorter (FACS), through manipulation of the forward scatter and side scatter settings. Other methods of separating cells based on physical properties include the use of filters with specific size ranges, as well as density gradients and pheresis techniques. When differentiation is desired based on electrical properties of cells, techniques such as electrophotoluminescence may be used in combination with a cell sorting means such as FACS. Selection of cells based on ability to uptake certain compounds can be performed using, for example, the ALDESORT system, which provides a fluorescent-based means of

purifying cells with high aldehyde dehydrogenase activity. Cells with high levels of this enzyme are known to possess higher proliferative and self-renewal activities in comparison to cells possessing lower levels. Other methods of identifying cells with high proliferative activity includes identifying cells with ability to selectively efflux certain dyes such as rhodamine-123 and or Hoechst 33342. Without being bound to theory, cells possessing this property often express the multidrug resistance transport protein ABCG2, and are known for enhanced regenerative ability compared to cells which do not possess this efflux mechanism. In other embodiments cord blood cells are purified for certain therapeutic properties based on expression of markers. In one particular embodiment, cord blood cells are purified for the phenotype of endothelial precursor cells. Said precursors, or progenitor cells express markers such as CD133, and/or CD34. Said progenitors may be purified by positive or negative selection using techniques such as magnetic activated cell sorting (MACS), affinity columns, FACS, panning, or by other means known in the art. Cord blood derived endothelial progenitor cells may be administered directly into the target tissue for obesity treatment, or may be administered systemically. Another variation of this embodiment is the use of differentiation of said endothelial precursor cells in vitro, followed by infusion into a patient. Verification for endothelial differentiation may be performed by assessing ability of cells to bind FITC-labeled Ulex europaeus agglutinin-1, ability to endocytose acetylated Di-LDL, and the expression of endothelial cell markers such as PECAM-1, VEGFR-2, or CD31.

[0060] Certain desired activities can be endowed onto said cord blood stem cells prior to administration into the patient. In one specific embodiment cord blood cells may be "activated" ex vivo by a brief culture in hypoxic conditions in order to upregulate nuclear translocation of the HIF-1 transcription factor and endow said cord blood cells with enhanced angiogenic potential. Hypoxia may be achieved by culture of cells in conditions of 0.1% oxygen to 10% oxygen, preferably between 0.5% oxygen and 5% oxygen, and more preferably around 1% oxygen. Cells may be cultured for a variety of timepoints ranging from 1 hour to 72 hours, more preferably from 13 hours to 59 hours and more preferably around 48 hours. Assessment of angiogenic, and other desired activities useful for the practice of the current invention, can be performed prior to administration of said cord blood cells into the patient.

[0061] In addition to induction of hypoxia, other therapeutic properties can be endowed unto cord blood stem cells through treatment ex vivo with factors such as de-differentiating compounds, proliferation inducing compounds, or compounds known to endow and/or enhance cord blood cells to possess properties useful for the practice of the current invention. In one embodiment cord blood cells are cultured with an inhibitor of the enzyme GSK-3 in order to enhance expansion of cells with pluripotent characteristics while not increasing the rate of differentiation. In another embodiment, cord blood cells are cultured in the presence of a DNA methyltransferase inhibitor such as 5-azacytidine in order to endow a "de-differentiation" effect. In another embodiment cord blood cells are cultured in the presence of a differentiation agent that skews said cord blood stem cells to generate enhance numbers of cells which are useful for treatment of obesity or for preventing secondary complications associated with obesity after said cord blood cells are administered into a patient. For example, cord blood cells may be cultured in testosterone for a brief period so that subsequent to administration, an increased number of cavernousal smooth muscle cells are generated in the patient in need thereof.

Placental stem cells

[0062] In contrast to cord blood stem cells, placental stem cells may be purified directly from placental tissues, said tissues including the chorion, amnion, and villous stroma. In another embodiment of the invention, placental tissue is mechanically degraded in a sterile manner and treated with enzymes to allow dissociation of the cells from the extracellular matrix. Such enzymes include, but not restricted to trypsin, chymotrypsin, collagenases, elastase and/or hylauronidase. Suspensions of placental cells are subsequently washed, assessed for viability, and may either be used directly for the practice of the invention by administration either locally or systemically. Alternatively, cells may be purified for certain populations with increased biological activity. Purification may be performed using means known in the art, and described above for purification of cord blood stem cells, or may be achieved by positive selection for the following markers: SSEA3, SSEA4, TRA1-60, TRA1-81, c-kit, and Thy-1. In some situations it will be desirable to expand cells before introduction into the human body. Expansion can be performed by culture ex vivo with

specific growth factors. The various embodiments of the invention described above for cord blood and embryonic stem cells can also be applied for placental stem cells.

Bone marrow stem cells

[0063] Bone marrow stem cells may be used either freshly isolated, purified, or subsequent to ex vivo culture. A typical bone marrow harvest for collecting starting material for practicing one embodiment of the invention involves a bone marrow harvest with the goal of acquiring approximately 5-700 ml of bone marrow aspirate. Numerous techniques for the aspiration of marrow are described in the art and part of standard medical practice. One particular methodology that may be attractive due to decreased invasiveness is the "mini-bone marrow harvest". Said aspirate is used as a starting material for purification of cells with therapeutic activity. In one specific embodiment bone marrow mononuclear cells are isolated by pheresis or gradient centrifugation. Numerous methods of separating mononuclear cells from bone marrow are known in the art and include density gradients such as Ficoll Histopaque at a density of approximately 1.077g/ml or Percoll gradient. Separation of cells by density gradients is usually performed by centrifugation at approximately 450g for approximately 25-60 minutes. Cells may subsequently be washed to remove debris and unwanted materials. Said washing step may be performed in phosphate buffered saline at physiological pH. An alternative method for purification of mononuclear cells involves the use of apheresis apparatus such as the CS3000-Plus blood-cell separator (Baxter, Deerfield, USA), the Haemonetics separator (Braintree, Mass), or the Fresenius AS 104 and the Fresenius AS TEC 104 (Fresenius, Bad Homburg, Germany) separators. In addition to injection of mononuclear cells, purified bone marrow subpopulations may be used. Additionally, ex vivo expansion and/or selection may also be utilized for augmentation of desired biological properties for use in treatment of obesity or for preventing secondary complications associated with obesity. The various embodiments of the invention described above for cord blood and embryonic stem cells can also be applied for bone marrow stem cells.

Amniotic fluid stem cells

[0064] Amniotic fluid is routinely collected during amniocentesis procedures. One method of practicing the current invention is utilizing amniotic fluid derived stem cells for treatment of obesity or for preventing secondary complications associated with obesity. In one embodiment amniotic fluid mononuclear cells are utilized therapeutically in an unpurified manner. Said amniotic fluid stem cells are administered either locally or systemically in a patient suffering from obesity. In other embodiments amniotic fluid stem cells are substantially purified based on expression of markers such as SSEA-3, SSEA4, Tra-1-60, Tra-1-81 and Tra-2-54, and subsequently administered. In other embodiments cells are cultured, as described in US Patent Application No. 2005/0054093 (incorporated by reference herein in its entirety), expanded, and subsequently infused into the patient. Amniotic stem cells are described in the following references. One particular aspect of amniotic stem cells that makes them amenable for use in practicing certain aspects of the current invention is their bi-phenotypic profile as being both mesenchymal and neural progenitors. The various embodiments of the invention described above for cord blood and embryonic stem cells can also be applied for amniotic fluid stem cells.

Neuronal stem cells

[0065] Stem cells committed to the neuronal lineage, or neuronal progenitor cells, are used in the practice of some specific embodiments of the invention. Said cells may be generated by differentiation of embryonic stem cells, may be freshly isolated from fetal tissue (ie mesencephalic), may be generated by transdifferentiation, or by reprogramming of a cell. Neuronal progenitors are selected by use of markers such as polysialyated N-CAM, N-CAM, A2B5, nestin and vimentin. The various embodiments of the invention described above for cord blood and embryonic stem cells can also be applied for neuronal stem cells.

Circulating peripheral blood stem cells

[0066] A wide variety of stem cells are known to circulate in the periphery. These include multipotent, pluripotent, and committed stem cells. In some embodiments of the invention mobilization of stem cells is induced in order to increase the number of circulating stem cells, so that harvesting efficiency is increased. Said mobilization allows for

harvest of cells with desired properties for practice of the invention without the need to perform bone marrow puncture. A variety of methods to induce mobilization are known. Methods such as administration of cytotoxic chemotherapy, for example, cyclophosphamide or 5-fluoruracil are effective but not preferred in the context of the current invention due to relatively unacceptable adverse events profile. Suitable agents useful for mobilization include: granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin 1 (IL-1), interleukin 3 (IL-3), stem cell factor (SCF, also known as steel factor or kit ligand), vascular endothelial growth factor (VEGF), Flt-3 ligand, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor-1 (FGF-1), fibroblast growth factor-2 (FGF-2), thrombopoietin (TPO), interleukin-11 (IL-11), insulin-like growth factor-1 (IGF-1), megakaryocyte growth and development factor (MGDF), nerve growth factor (NGF), hyperbaric oxygen, and 3-hydroxy-3-methyl glutaryl coenzyme A (HMG CoA)reductase inhibitors. The various embodiments of the invention described above for cord blood and embryonic stem cells can also be applied for circulating peripheral blood stem cells.

[0067] In a preferred embodiment, donors (either autologous or allegeneic) are mobilized by administration of G-CSF (filgrastim: neupogen) at a concentration of 10ug/kg/day by subcutaneous injection for 2-7 days, more preferably 4-5 days. Peripheral blood mononuclear cells are collected using an apheresis device such as the AS104 cell separator (Fresenius Medical). 1-40 x 10^9 mononuclear cells are collected, concentrated and injected into the area of interest in an intramuscular manner. Alternatively, cells may be injected systemically. Additionally cells may be purified for specific subtypes before and/or after culture. Treatments can be made to the cells during culture or at specific timepoints during ex vivo culture but before infusion in order to generate and/or expand specific subtypes and/or functional properties.

Mesenchymal stem cells

[0068] In one embodiment mesenchymal cells are generated through culture. For example, U.S. Patent No. 5,486,359 (incorporated by reference herein in its entirety) describes methods for culturing such and expanding mesenchymal stem cells, as well as

providing antibodies for use in detection and isolation. U.S. Patent No. 5,942,225 (incorporated by reference herein in its entirety) teaches culture techniques and additives for differentiation of such stem cells which can be used in the context of the present invention to produce increased numbers of cells with angiogenic capability. Although U.S. Patent No. 6,387,369 (incorporated by reference herein in its entirety) teaches use of mesenchymal stem cells for regeneration of cardiac tissue, in accordance with published literature, stem cells generated through these means are actually angiogenically potent and therefore may be utilized in the context of the current invention for treatment/amelioration of obesity or for preventing secondary complications associated with obesity

[0069] Mesenchymal stem cells are classically obtained from bone marrow sources for clinical use, although this source may have disadvantages because of the invasiveness of the donation procedure and the reported decline in number of bone marrow derived mesenchymal stem cells during aging. Alternative sources of mesenchymal stem cells include adipose tissue, placenta, scalp tissue and cord blood. A recent study compared mesenchymal stem cells from bone marrow, cord blood and adipose tissue in terms of colony formation activity, expansion potential and immunophenotype. It was demonstrated that all three sources produced mesenchymal stem cells with similar morphology and phenotype. Ability to induce colony formation was highest using stem cells from adipose tissue and interestingly in contrast to bone marrow and adipose derived mesenchymal cells, only the cord blood derived cells lacked ability to undergo adipocyte differentiation. Proliferative potential was the highest with cord blood mesenchymal stem cells which were capable of expansion to approximately 20 times, whereas adipose derived mesenchymal cells expanded an average of 8 times and bone marrow derived cells expanded 5 times. Accordingly, one skilled in the art will understand that mesenchymal stem cells for use with the present invention may be selected upon individual patient characteristics and the end result sought. For example, if autologous mesenchymal stem cells are available in the form of adipocyte-derived cells, it will be useful to utilize this source instead of allogeneic cord-blood derived cells. Alternatively, cord blood derived mesenchymal stem cells may be more advantageous for use in situations where autologous cells are not available, and expansion is sought.

[0070] The ability of mesenchymal stem cells from the cord blood to expand in vitro also allows the possibility of genetically modifying these cells in order to: a) decrease immunogeneicity; b) enhance angiogenic potential; and c) augment survival following administration. However it should be noted that such *ex vivo* manipulation is applicable to all cell types described in the current application.

[0071] In situations where a decrease in immunogenicity is sought, cells may be transfected using immune suppressive agents. Said agents include soluble factors, membrane-bound factors, and enzymes capable of causing localized immune suppression. Examples of soluble immune suppressive factors include: IL-4, IL-10, IL-13, TGF-b, soluble TNF-receptor, and IL-1 receptor agonist. Membrane-bound immunoinhibitor molecules that may be transfected into stem cells for use in practicing the current invention include: HLA-G, FasL, PD-1L, Decay Accelerating Factor, and membrane-associated TGF-b. Enzymes which may be transfected in order to cause localized immune suppression include indolamine 2,3 dioxygenase and arginase type II. In order to optimize desired immune suppressive ability, a wide variety of assays are known in the art, including mixed lymphocyte culture, ability to generate T regulatory cells in vitro, and ability to inhibit natural killer or CD8 cell cytotoxicity.

[0072] In situations where increased angiogenic potential of said mesenchymal stem cells is desired, mesenchymal stem cells may be transfected with genes such as VEGF, FGF1, FGF2, FGF4, FrzA, and angiopoietin. Ability to induce angiogenesis may be assessed *in vitro* prior to administration of said transfected cells *in vivo*. Methods of assessing *in vitro* angiogenesis stimulating ability are well known in the art and include measuring proliferation of human umbilical vein derived endothelial cells.

[0073] Since one of the problems of cell therapy in general is viability of the infused cells subsequent to administration, it may be desired in some forms of the invention to transfect mesenchymal cells with genes protecting said cells from apoptosis. Anti-apoptotic genes suitable for transfection may include bcl-2, bcl-xl, and members of the XIAP family. Alternatively it may be desired to increase the proliferative lifespan of said mesenchymal stem cells through transfection with enzymes associated with anti-senescence activity. Said enzymes may include telomerase or histone deacetylases. The various

embodiments of the invention described above for cord blood and embryonic stem cells can also be applied for mesenchymal stem cells.

Adipose tissue derived stem cells

[0074] Adipose derived stem cells express markers such as CD9; CD29 (integrin beta 1); CD44 (hyaluronate receptor); CD49d,e (integrin alpha 4, 5); CD55 (decay accelerating factor); CD105 (endoglin); CD106 (VCAM-1); CD166 (ALCAM). These markers are useful not only for identification but may be used as a means of positive selection, before and/or after culture in order to increase purity of the desired cell population. In terms of purification and isolation, devices are known to those skilled in the art for rapid extraction and purification of cells adipose tissues. U.S. Patent No.6,316,247 (incorporated by reference herein in its entirety) describes a device which purifies mononuclear adipose derived stem cells in an enclosed environment without the need for setting up a GMP/GTP cell processing laboratory so that patients may be treated in a wide variety of settings. One embodiment of the invention involves attaining 10-200 ml of raw lipoaspirate, washing said lipoaspirate in phosphate buffered saline, digesting said lipoaspirate with 0.075% collagenase type I for 30-60 min at 37°C with gentle agitation, neutralizing said collagenase with DMEM or other medium containing autologous serum, preferably at a concentration of 10% v/v, centrifuging the treated lipoaspirate at approximately 700-2000g for 5-15 minutes, followed by resuspension of said cells in an appropriate medium such as DMEM. Cells are subsequently filtered using a cell strainer, for example a 100 μm nylon cell strainer in order to remove debris. Filtered cells are subsequently centrifuged again at approximately 700-2000g for 5-15 minutes and resuspended at a concentration of approximately $1x10^6/cm^2$ into culture flasks or similar vessels. After 10-20 hours of culture non-adherent cells are removed by washing with PBS and remaining cells are cultured at similar conditions as described above for culture of cord blood derived mesenchymal stem cells. Upon reaching a concentration desired for clinical use, cells are harvested, assessed for purity and administered in a patient in need thereof as described above. The various embodiments of the invention described above for cord blood and embryonic stem cells can also be applied for adipose derived stem cells.

Exfoliated teeth derived stem cells

[0075] Deciduous teeth (baby teeth) have been recently identified as a source of pluripotent stem cells with ability to differentiate into endothelial, neural, and bone structures. Said pluripotent stem cells have been termed "stem cells from human exfoliated deciduous teeth" (SHED). One of the embodiments of the current invention involves utilization of this novel source of stem cells for the treatment of obesity or for preventing secondary complications associated with obesity. In one embodiment of the invention, SHED cells are administered systemically or locally into a patient with obesity at a concentration and frequency sufficient for induction of therapeutic effect. SHED cells can be purified and used directly, certain sub-populations may be concentrated, or cells may be expanded ex vivo under distinct culture conditions in order to generate phenotypes desired for maximum therapeutic effect. Growth and expansion of SHED has been previously described by others. In one particular method, exfoliated human deciduous teeth are collected from 7- to 8-year-old children, with the pulp extracted and digested with a digestive enzyme such as collagenase type I. Concentrations necessary for digestion are known and may be, for example 1-5 mg/ml, or preferable around 3 mg/ml. Additionally dispase may also be used alone or in combination, concentrations of dispase may be 1-10 mg/ml, preferably around 4 mg/ml. Said digestion is allowed to occur for approximately 1 h at 37°C. Cells are subsequently washed and may be used directly, purified, or expanded in tissue culture. The various embodiments of the invention described above for cord blood and embryonic stem cells can also be applied for exfoliated teeth stem cells.

Hair follicle stem cells

[0076] The bulge area of the hair follicle bulge is an easily accessible source of pluripotent mesenchymal-like stem cells. One embodiment of the current invention is the use of hair follicle stem cells for treatment of obesity or for preventing secondary complications associated with obesity. Said cells may be used therapeutically once freshly isolated, or may be purified for particular sub-populations, or may be expanded *ex vivo* prior to use. Purification of hair follicle stem cells may be performed from cadavers, from healthy

volunteers, or from patients undergoing plastic surgery. Upon extraction, scalp specimens are rinsed in a wash solution such as phosphate buffered saline or Hanks and cut into sections 0.2-0.8 cm. Subcutaneous tissue is de-aggregated into a single cell suspension by use of enzymes such as dispase and/or collagenase. In one variant, scalp samples are incubated with 0.5% dispase for a period of 15 hours. Subsequently, the dermal sheath is further enzymatically de-aggregated with enzymes such as collagenase D. Digestion of the stalk of the dermal papilla, the source of stem cells is confirmed by visual microscopy. Single cell suspensions are then treated with media containing fetal calf serum, and concentrated by pelletting using centrifugation. Cells may be further purified for expression of markers such as CD34, which are associated with enhanced proliferative ability. In one embodiment of the invention, collected hair follicle stem cells are induced to differentiate in vitro into neural-like cells through culture in media containing factors such as FGF-1, FGF-2, NGF, neurotrophin-2, and/or BDNF. Confirmation of neural differentiation may be performed by assessment of markers such as Muhashi, polysialyated N-CAM, N-CAM, A2B5, nestin, vimentin glutamate, synaptophysin, glutamic acid decarboxylase, serotonin , tyrosine hydroxylase, and GABA. Said neuronal cells may be administered systemically, or locally in a patient being treated for obesity or for prevention of secondary complications associated with obesity. The various embodiments of the invention described above for cord blood and embryonic stem cells can also be applied for hair follicle stem cells.

Parthenogenically derived stem cells

[0077] Parthenogenically derived stem cells can be generated by addition of a calcium flux inducing agent to activate oocytes, followed by purifying and expanding cells expressing embryonic stem cell markers such as SSEA-4, TRA 1-60 and/or TRA 1-81. Said parthenogenically derived stem cells are totipotent and can be used in a manner similar to that described for embryonic stem cells in the practice of the current invention. The various embodiments of the invention described above for cord blood and embryonic stem cells can also be applied for parthenogenically derived stem cells.

Reprogrammed stem cells

[0078] Reprogramming of non-stem cells to endow them with stem cell characteristics can generate stem cells for use in the practice of the current invention. The advantage of reprogramming cells is that ability to withdraw autologous cells, which may have limited stem cell potential, endow said autologous cells with stem cell, or stem cell-like, properties, and reintroduce said autologous cells into the patient. The various embodiments of the invention described above for cord blood and embryonic stem cells can also be applied for reprogrammed stem cells.

Side population stem cell

[0079] Cells expressing the ability to efflux certain dyes, including but not limited to rhodamin-123 are associated with stem cell-like properties. Said cells can be purified from tissue subsequent to cell dissociation, based on efflux properties. Accordingly, in one embodiment of the current invention, tissue derived side population cells may be utilized either freshly isolated, sorted into subpopulations, or subsequent to ex vivo culture, for the treatment of obesity or for preventing secondary complications associated with obesity. For use in the invention, side population cells may be derived from tissues such as pancreatic tissue, liver tissue, smooth muscle tissue, striated muscle tissue, cardiac muscle tissue, bone tissue, bone marrow tissue, bone spongy tissue, cartilage tissue, liver tissue, pancreas tissue, pancreatic ductal tissue, spleen tissue, thymus tissue, Peyer's patch tissue, lymph nodes tissue, thyroid tissue, epidermis tissue, dermis tissue, subcutaneous tissue, heart tissue, lung tissue, vascular tissue, endothelial tissue, blood cells, bladder tissue, kidney tissue, digestive tract tissue, esophagus tissue, stomach tissue, small intestine tissue, large intestine tissue, adipose tissue, uterus tissue, eye tissue, lung tissue, testicular tissue, ovarian tissue, prostate tissue, connective tissue, endocrine tissue, and mesentery tissue. More optimally, side population cells obtained from smooth muscle tissue are administered at a concentration and frequency sufficient to induce a therapeutic effect on obesity or for preventing secondary complications associated with obesity. The various embodiments of the invention described above for cord blood and embryonic stem cells can also be applied for side population stem cells.

Routes of administration

[0080] Routes of administration of stem cells are known in the art and may include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., ingestion or inhalation), transdermal (topical), transmucosal, and rectal administration.

[0081] One simple method of administering stem cells is through the systemic route. Systemic administration of stem cells requires dilution of cells into appropriate solutions so that cells maintain viability. In one embodiment of the invention cells are administered in a solution of phosphate buffered saline, in another embodiment cells are dissolved in a solution of saline supplemented with autologous serum at a concentration ranging between 1-10%, preferably, between 2-7%, and even more preferably at a concentration of approximately 3%. It is known to one skilled in the art that various concentrations of albumin may also be added with the saline for injection of cells. Ideally pH of the injection solution should be from about 6.4 to about 8.3, optimally 7.4. Excipients may be used to bring the solution to isotonicity such as, 4.5% mannitol or 0.9% sodium chloride, pH buffers with art-known buffer solutions. Other pharmaceutically acceptable agents can also be used to bring the solution to isotonicity, including, but not limited to, dextrose, boric acid, sodium tartrate, propylene glycol, polyols (such as mannitol and sorbitol) or other inorganic or organic solutes.

[0082] Concentration and frequency of cellular administration is dependent on patient characteristics, as well as type of stem cells used. Numerous other factors may be used to guide the practitioner of the invention for adjusting the dose of stem cells administered. Said factors include the amount of endogenous stem cells circulating in the patient, the activity of stem cells in the patient (ie proliferative, colony formation, chemotactic mobility, etc), and the degree of the target indication that is observed in the patient.

Injection steps and dose ranges

[0083] In any of the above embodiments where an agent capable of mobilizing endogenous stem cells is administered, a range of effective concentrations can be used. For example, where G-CSF is administered, G-CSF can be administered at a concentration

ranging from about 0.01, about 0.1, about 1 to about 5000, about 2000, about 1000, about 900, about 800, about 700, about 600, about 500, about 400, about 300, about 200, about 100, about 50, about 25, about 10, 9, 8, 7, 6, 5, 4, 3, or about 2 micrograms/kilogram of patient body weight per day for a period ranging from about 1 day to about 100 days. Other similar dose ranges are applicable for agent such as: M-CSF, G-CSF, GM-CSF, an antagonist of CXCR-4, an antagonist of VLA-4, fucoidan, IVIG, parathyroid hormone, and cyclophosphamide.

[0084] In any of the above embodiments, the amount of purified mononuclear cells administered can be at least 1, at least 10, at least 100, at least 1,000, at least 10,000, at least 100,000, at least 1 million, at least 10 million, or at least 100 million cells. The amount of cells administered can range from 1 cell to about 100 million cells, from about 100 cells to about 10 million cells, from about 1000 cells to about 10 million cells, from about 10,000 cells to about 10 million cells, from about 100,000 cells to about 10 million cells, from about 1 million cells to about 10 million cells, and from about 1 million cells to about 5 million cells.

[0085] In one embodiment of the invention, mononuclear cells are concentrated in an injection solution, which may be saline, mixtures of autologous plasma together with saline, or various concentrations of albumin with saline. Typically the pH of the injection solution is from about 6.4 to about 8.3, optimally 7.4. Excipients may be used to bring the solution to isotonicity such as, 4.5% mannitol or 0.9% sodium chloride, pH buffers with art-known buffer solutions, such as sodium phosphate. Other pharmaceutically acceptable agents can also be used to bring the solution to isotonicity, including, but not limited to, dextrose, boric acid, sodium tartrate, propylene glycol, polyols (such as mannitol and sorbitol) or other inorganic or organic solutes.

EXAMPLES

EXAMPLE 1

INDUCTION OF WEIGHT LOSS BY AUTOLOGOUS ADIPOSE DERIVED MONONUCLEAR CELLS

[0086] A clinical trial is conducted with obese patients (BMI greater or equal to 40). About half of the patients are placed in the control group and receive placebo, while the other half of the patients undergo treatment. All patients, including control, receive liposuction. Patients in the treated group are administered mononuclear cells extracted from said lipoaspirate. Mononuclear cells are prepared according to standard protocols, briefly, lipoaspirate is washed in phosphate buffered saline and digested with 0.075% collagenase type I for 30-60 min at 37°C by gentle agitation. After the digestion is completed neutralization of said collagenase with DMEM or other medium containing autologous serum is performed, preferably at a concentration of 10% v/v autologous serum to media. Said cells are centrifuged at approximately 700-2000g for 5-15 minutes, followed by resuspension of said cells in an appropriate medium such as DMEM. Cells are then filtered using a cell strainer, for example a 100 μm nylon cell strainer in order to remove debris. Filtered cells are subsequently centrifuged again at approximately 700-2000g for 5-15 minutes and administered intravenously. On average each of the treated patients had 50 ml of lipoaspirate processed. 1 month after treatment patients have a reduction in overall weight as well as in the BMI whereas control patients which received placebo injections do not have alterations in weight or BMI.

EXAMPLE 2

INDUCTION OF WEIGHT LOSS BY AUTOLOGOUS BONE MARROW DERIVED STEM CELLS

[0087] A clinical trial is conducted with obese patients (BMI greater or equal to 40). About half of the patients are placed in the control group and receive placebo, while the other half of the patients undergo treatment. All patients, including control, undergo bone

marrow harvest. Patients in the treated group are administered bone marrow stem cells whereas control patients are administered placebo. Patients are subjected to a bone marrow harvest. Briefly, patients are positioned face down on a horizontal platform and provided analgesics as per standard medical procedures. All personnel involved in the procedure is dressed in sterile surgical gowning and masks. The harvesting field consisting of both iliac crests is prepared by topically applying standard disinfectant solution. Iliac crests are anaesthetized and the harvesting needle is inserted in order to puncture the iliac crest. The cap and stylet of the harvesting needle is removed and 3-ml of marrow is harvested into the 15-ml harvesting syringe containing heparin solution. The process is repeated and the contents of the harvesting syringe are transferred into a 500-ml collecting bag. Approximately 75-125 ml of bone marrow is harvested in total.

[0088] Isolation of mononuclear cells is performed by gradient separation using the Hetastarch method, which is clinically applicable and reported to remove not only erythrocytes but also granulocytic cells. Six-percent (wt/vol) Hetastarch (HES40, Hishiyama Pharmaceutical Co., Osaka, Japan) is added to the collected bone marrow sample to achieve a final concentration of 1.2 percent Hetastarch, (1:5 volume ratio of added Hetastarch to bone marrow). Centrifugation at 50g for 5 min at 10°C is performed in order to generate a leukocyte rich supernatant. Sedimentation of bone marrow takes place at a cell concentration of no more than 15 x 10^6 cells/ml in a total volume of 850 ml per Hetastarch bag. The supernatant is transferred into a plasma transfer bag and centrifuged (400g for 10 min) to sediment the cells. The sedimented cells are subsequently washed in phosphate buffered saline in the presence of 5% penicillin/streptomycin mixture (Gibco, Mississauga, Canada) and 5% autologous serum. Cellular viability and lack of potential contamination with other cells is assessed by microscopy. Bone marrow mononuclear cells are administered into patients in the treated group. On average each of the treated patients had 75-125 ml of bone marrow processed. 1 month after treatment patients had a reduction in overall weight as well as in the BMI whereas control patients which received placebo injections did not have alterations in weight or BMI.

WHAT IS CLAIMED IS:

1. A method of treating obesity in a subject comprising the steps of: a) selecting a patient in need of treatment for obesity; and b) administering a therapeutically effective amount of purified mononuclear cells to said patient.

2. The method of claim 1, wherein said mononuclear cells have been isolated from tissue of said patient.

3. A method of preventing secondary complications associated with obesity comprising the steps of: a) selecting a patient in need of treatment for secondary complications associated with obesity; and b) administering a therapeutically effective amount of purified mononuclear cells to said patient.

4. The method of claim 3, wherein said mononuclear cells have been isolated from tissue of said patient.

5. A method of treating obesity in a subject comprising the steps of: a) withdrawing an amount of adipose tissue from said subject; b) substantially purifying mononuclear cells from said adipose tissue; and c) administering a therapeutically effective amount of said purified mononuclear cells to said subject.

6. The method of any of claims 1-5 wherein said purified mononuclear cells are substantially free of adipocytes.

7. The method of any of claims 1-5, wherein said purified mononuclear cells are administered systemically.

8. The method of any of claims 1-5, wherein subsequent to isolation of said substantially purified mononuclear cells, said cells are divided into aliquots, with some aliquots being frozen for future administration, while some aliquots are administered to said subject after purification.

9. The method of any of claims 1-5, wherein the number of purified mononuclear cells administered to said patient is adjusted based on extent of desired weight loss.

10. The method of any of claims 1-5, wherein said number of purified mononuclear cells administered to said patient is adjusted based on modification of metabolic parameters.

11. The method of any of claims 1-5, wherein said obesity is associated with a metabolic dysfunction.

12. The method of claim 11, wherein said metabolic dysfunction is associated or caused by a condition selected from the group of the following conditions: Prader-Willi syndrome, Cushing syndrome, drug-induced metabolic dysfunction, congenital metabolic dysfunctions, hypothyroidism and growth hormone deficiency.

13. A method of preventing secondary complications associated with obesity comprising the steps of: a) withdrawing an amount of adipose tissue from said subject; b) substantially purifying mononuclear cells from said adipose tissue; and c) reintroducing a therapeutically effective amount of purified mononuclear cells to said subject.

14. The method of claim 13 wherein purified mononuclear cells are substantially free of adipocytes.

15. The method of claim 13, wherein said purified mononuclear cells are administered systemically.

16. The method of claim 13, wherein subsequent to isolation of said substantially purified mononuclear cells, said cells are divided into aliquots, with some aliquots being frozen for future administration, while some aliquots are administered to said subject after purification.

17. The method of claim 13, wherein the number and/or location of purified mononuclear cells re-introduced into said patient is adjusted based on extent of desired protective and/or therapeutic effect on said secondary complication associated with obesity.

18. The method of claim 13, wherein said secondary complication of obesity is selected from a group consisting of: atherosclerosis, endothelial dysfunction, coronary artery disease, insulin resistance, cor pulmonale, pulmonary hypertension, left ventricular hypertrophy, cardiomyopathy, dyslipidemia, obstructive sleep apnea, systemic hypertension, renal failure, stress incontinence, fatty liver disease, and predisposition to stroke.

19. A method of treating obesity in a subject comprising the steps of: a) selecting a stem cell source; b) purifying and/or concentrating stem cells from said stem cell source; and c) reintroducing a therapeutically effective amount of purified mononuclear cells to said subject.

20. The method of claim 19, wherein said stem cell source is selected from a group consisting of: autologous, allogeneic, and xenogeneic.

21. The method of claim 16, wherein said stem cell source is selected from a group consisting of: bone marrow, peripheral blood, mobilized peripheral blood, cord blood, menstrual blood, wharton's jelly, placental matrix, embryonic, fetal, retrodifferentiated tissue, side population cells, amnion, amniotic fluid, deciduous tooth, hair follicle, paraventricular zone, and adipose tissue.

22. The method of claim 20 wherein purified mononuclear cells are extracted from said stem cell source and administered into a patient in need thereof as a heterogenous population containing stem cells and other mononuclear cells specific to said tissue.

23. The method of claim 22, wherein said purified mononuclear cells are enriched for cells with stem cell activity.

24. The method of claim 23, wherein said cells with stem cell activity are enriched by a method selected from a group of methods consisting of: a) selection based on P-glycoprotein drug efflux pump activity as measured by extrusion of a substrate, said representative substrate being rhodamine 123; b) selection based on multidrug resistance protein-1 activity as measured by extrusion of a substrate, said representative substrate being Fluo-3; c) selection based on aldehyde dehydrogenase activity as measured by ALDEFLUOR staining; d) selection based on expression of CD117; e) selection based on expression of CD133; f) selection based on expression of CD34; and g) selection based on markers associated with high replicative potential cells.

25. The method of claim 19 wherein said purified mononuclear cells are substantially free of non-stem cells.

26. The method of claim 19, wherein said purified mononuclear cells are administered systemically.

27. The method of claim 19, wherein subsequent to isolation of said substantially purified mononuclear cells, said cells are divided into aliquots, with some aliquots being frozen for future administration, while some aliquots are administered to said subject after purification.

28. The method of claim 19, wherein the number and/or location of purified mononuclear cells re-introduced into said patient is adjusted based on extent of desired protective and/or therapeutic effect on said secondary complication associated with obesity.

29. A method of preventing secondary complications associated with obesity comprising the steps of: a) selecting a stem cell source; b) purifying and/or concentrating stem cells from said stem cell source; and c) reintroducing a therapeutically effective amount of purified mononuclear cells to said subject.

30. The method of claim 29, wherein said stem cell source is selected from a group consisting of: autologous, allogeneic, and xenogeneic.

31. The method of claim 29, wherein said stem cell source is selected from a group consisting of: bone marrow, peripheral blood, mobilized peripheral blood, cord blood, menstrual blood, wharton's jelly, placental matrix, embryonic, fetal, retrodifferentiated tissue, side population cells, amnion, amniotic fluid, deciduous tooth, hair follicle, paraventricular zone, and adipose tissue.

32. The method of claim 31 wherein purified mononuclear cells are extracted from said stem cell source and administered into a patient in need thereof as a heterogenous population containing stem cells and other mononuclear cells specific to said tissue.

33. The method of claim 32, wherein said purified mononuclear cells are enriched for cells with stem cell activity.

34. The method of claim 33, wherein said cells with stem cell activity are enriched by a method selected from a group of methods consisting of: a) selection based on P-glycoprotein drug efflux pump activity as measured by extrusion of a substrate, said representative substrate being rhodamine 123; b) selection based on multidrug resistance protein-1 activity as measured by extrusion of a substrate, said representative substrate being Fluo-3; c) selection based on aldehyde dehydrogenase activity as measured by ALDEFLUOR staining; d) selection based on expression of CD117; e) selection based on expression of CD133; f) selection based on expression of CD34; and g) selection based on markers associated with high replicative potential cells.

35. The method of claim 29 wherein said purified mononuclear cells are substantially free of non-stem cells.

36. The method of claim 29, wherein said purified mononuclear cells are administered systemically.

37. The method of claim 29, wherein subsequent to isolation of said substantially purified mononuclear cells, said cells are divided into aliquots, with some aliquots being frozen for future administration, while some aliquots are administered to said subject after purification.

38. The method of claim 29, wherein the number and/or location of purified mononuclear cells re-introduced into said patient is adjusted based on extent of desired protective and/or therapeutic effect on said secondary complication associated with obesity.

39. The method of claim 29, wherein said secondary complication of obesity is selected from a group consisting of: atherosclerosis, endothelial dysfunction, coronary artery disease, insulin resistance, cor pulmonale, pulmonary hypertension, left ventricular hypertrophy, cardiomyopathy, dyslipidemia, obstructive sleep apnea, systemic hypertension, renal failure, stress incontinence, fatty liver disease, and predisposition to stroke.

40. A method of inducing weight loss in a subject through the steps of claims 5 and 19.

41. A method of increasing efficacy of a therapy or intervention that induces weight loss through the steps of claims 5 and 19.

42. A method of a method of treating obesity in a subject comprising providing a therapeutically effective amount of purified mononuclear cells to a patient in need of or selected for treatment for obesity.

43. A method of preventing secondary complications associated with obesity in a subject comprising providing a therapeutically effective amount of purified mononuclear cells to a patient in need of or selected for treatment for secondary complications associated with obesity.

44. The method of any of the above claims, wherein said purified mononuclear cells administered is in an amount ranging from 1 cell to about 100 million cells.

45. The method of claim 44, wherein said purified mononuclear cells administered is in an amount ranging from about 100 cells to about 10 million cells.

46. The method of claim 44, wherein said purified mononuclear cells administered is in an amount ranging from about 10,000 cells to about 10 million cells.

47. The method of claim 44, wherein said purified mononuclear cells administered is in an amount ranging from about 100,000 cells to about 10 million cells.

48. The method of claim 44, wherein said purified mononuclear cells administered is in an amount ranging from about 1 million cells to about 10 million cells.