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(54) **Title**: ERYTHROPOIETIN-EXPRESSING ADIPOSE CELL POPULATIONS

(57) **Abstract**: The present invention relates to isolated cell populations derived from adipose tissue that express erythropoietin (EPO).

ERYTHROPOIETIN-EXPRESSING ADIPOSE CELL POPULATIONS

Field of the Invention

The present invention relates to isolated cell populations derived from adipose tissue that express erythropoietin (EPO).

Background of the Invention

Chronic Kidney Disease (CKD) affects over 19M people in the United States and is frequently a consequence of metabolic disorders involving obesity, diabetes, and hypertension. Examination of the data reveals that the rate of increase is due to the development of renal failure secondary to hypertension and non-insulin dependent diabetes mellitus (NIDDM) (United States Renal Data System: Costs of CKD and ESRD. ed. Bethesda, MD, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2007 pp 223-238) – two diseases that are also on the rise worldwide. CKD patients in stages 1-3 of progression are managed by lifestyle changes and pharmacological interventions aimed at controlling the underlying disease state(s), while patients in stages 4-5 are managed by dialysis and a drug regimen that typically includes anti-hypertensive agents, erythropoiesis stimulating agents (ESAs), iron and vitamin D supplementation. Chronic renal failure is prevalent in humans as well as some domesticated animals. Patients with renal failure experience not only the loss of kidney function (uremia), but also develop anemia due to the inability of the bone marrow to produce a sufficient number of red blood cells (RBCs) via erythropoiesis. Erythroid homeostasis is dependent on both the production of erythropoietin (EPO) by specialized interstitial fibroblasts that reside in the kidney and the ability of targeted erythroid progenitors in the bone marrow to respond to EPO and manufacture more RBCs. The anemia of renal failure is due to both reduced production of EPO in the kidney and the negative effects of uremic factors on the actions of EPO in the bone marrow.

To date, clinical approaches to the treatment of chronic renal failure involve dialysis and kidney transplantation for restoration of renal filtration and urine production, and the systemic delivery of recombinant EPO or EPO analogs to restore erythroid mass. Preclinical investigations have examined in vivo efficacy and safety of EPO-producing cells that have been generated via gene therapy approaches. However, new treatment paradigms are needed that provide substantial and durable augmentation of kidney functions, to slow disease progression and improve quality of life in this patient population and reduce the annual cost burden on the healthcare system. Regenerative medicine technologies may provide next-generation therapeutic options for CKD. Adipose is recognized as an endocrine organ with significant metabolic bioactivity. Adipose tissue is composed of adipocytes, vascular endothelial cells, pericytes, fibroblasts, macrophages, stem cells and progenitors with MSC-like bioactivity and smooth muscle-like cells (Crisan, M. et al. Cell Stem Cell **3**, 301, 2008; da Silva Meirelles, L. et al. J Cell Sci **119**, 2204, 2006; Lin, G. et al. Stem Cells Dev **17**, 1053, 2008; Basu, J. et al. Tissue Eng Part C, In Press, 2011). Of these, MSC-like and smooth muscle-like cell populations are currently under active development for application in tissue engineering and regenerative medicine (Basu, J. and Ludlow, J.W.

Trends Biotechnol **28**, 526, 2010; Basu, J. et al. Tissue Eng Part C Methods, 2011 May 19 [Epub ahead of print). At a higher level, adipose tissue may be classified as white or brown based on the preponderance of white or brown adipocytes. White adipocytes represent the principal lipid storage vehicle within adipose tissue, whereas brown adipocytes are responsible for mediating lipid metabolism and are therefore correspondingly enriched in mitochondria. Adipose tissue may be found distributed broadly throughout the body as distinctive, region specific depots. The principal depots for white adipose tissue (WAT) are abdominal subcutaneous and visceral adipose tissue (SAT and VAT). VAT may itself be further subdivided into omental, mesenteric, retroperitoneal, gonadal and pericardial depots (Bjorndal, B. et al. J Obes, 2011, Volume 2011, Article ID 490650, 15 pages; Cook, A. and Cowan, C., Adipose (March 31, 2009), StemBook, ed. The Stem Cell Research Community). Adipose depots are characterized by unique patterns of structural organization, transcriptomic, proteomic and secretomic expression profiles and biological function. For example, secretomes generated by visceral, subcutaneous and gonadal adipose depots are specific to source (Roca-Rivada, A. et al. J Proteomics 74(7): 1068-1079, 2011). Furthermore, significant functional differences between subcutaneous, epididymal and mesenteric adiposes have been observed through transcriptomic and lipidomic analysis of transgenic mice with humanized lipoprotein profiles (Caesar, R. et al. PLoS One 5(7):e11525, 2010). Finally, the multi-lineage differentiation potential of adipose-derived stromal cells with MSC-like bioactivity has been shown to be dependent on the depot of origin (Levi, B. et al. Plast Reconstr Surg **126**, 822, 2010; Schipper, B.M. et al. Ann Plast Surg **60**, 538, 2008). These systemic observations notwithstanding, analysis and characterization of transcriptomic, proteomic and functional differences between adiposes associated with individual organs remains to be investigated. More specifically, understanding the variation in regenerative potentials presented by stromal cells derived from differently sourced solid organ associated adipose may significantly impact the development of tissue engineering and regenerative medicine (TE/RM) products targeted to those organs.

Summary of the Invention

The present invention relates to cell populations derived from adipose tissue obtained from any suitable adipose source in the body. In one embodiment, the cell population is an isolated adipose cell population which expresses erythropoietin (EPO). Suitable sources of adipose tissue include, without limitation, heart adipose, liver adipose, subcutaneous adipose, abdominal or visceral adipose, white adipose, brown adipose, and renal adipose. In one embodiment, the EPO-expressing cell population may be derived from renal adipose tissue. The cell population may be derived from renal pedicle adipose tissue and/or renal calyx adipose tissue. In one other embodiment, the cell population may be derived from an adipose stromal vascular fraction (SVF).

In another embodiment, the cell populations further express VEGF. The expression of EPO and/or VEGF is hypoxia-regulated expression. In one other embodiment, the EPO transcript and/or EPO polypeptide are expressed by the cell population. In another embodiment, the VEGF transcript and/or VEGF polypeptide is expressed by the cell population.

In one other embodiment, the EPO polypeptide expressed by an adipose-derived cell population is characterized by post-translational modification that is different from a non-adipose cell population. The non-adipose cell population may be selected from the group consisting of keratinocytes, hepatocytes, and primary kidney cells.

In another embodiment, the EPO polypeptide expressed by a renal adipose-derived cell population is characterized by post-translational modification that is different from a non-renal adipose cell population. The non-renal adipose cell population may be selected from the group consisting of keratinocytes, hepatocytes, visceral adipose stromal cell, and primary kidney cells.

In some embodiments, the renal adipose-derived cell population differentially expresses a biomarker associated with renal regeneration. The differential biomarker expression may be increased expression. The biomarker may be WT-1. The WT-1 biomarker may be a WT-1 transcript may be expressed, including a KTS^+ and/or KTS^- transcript. The biomarker may be a WT-1 polypeptide.

In another aspect, the present invention provides a method of preparing an erythropoietin (EPO)-expressing adipose stromal cell population. In one embodiment, the method includes the step of digesting adipose tissue. The method may further include the step of depleting the digested tissue of adipocytes to provide a stromal vascular fraction (SVF). The depletion step may be performed after the digestion step. The SVF will contain the EPO-expressing adipose stromal cell population. In one embodiment, the adipose tissue may be renal adipose tissue and the EPO-expressing renal adipose stromal cell population is a kidney sourced adipose stromal (KiSAS) cell population described herein.

In one other aspect, the present invention provides an implantable construct for providing improved kidney function to a subject in need. In one embodiment, the construct will include a biocompatible matrix. In another embodiment, the construct will also have an adipose cell population which express erythropoietin (EPO) deposited on or embedded in a surface of the matrix. In one other embodiment, the EPO-expressing adipose stromal cell population is the KiSAS cell population described herein.

In yet another aspect, the present invention provides methods of treating a kidney disease in a subject in need. In one embodiment, the method includes the step of administering to the subject a composition having an adipose cell population which expresses erythropoietin (EPO). In another embodiment, the EPO-expressing adipose stromal cell population is the KiSAS cell population described herein.

In one other aspect, the present invention provides methods of treating a kidney disease in a subject in need through the use of a construct. In one embodiment, the method includes the step of administering to the subject a construct. The construct may have a biocompatible matrix. In another embodiment, the construct may also have an adipose cell population which expresses erythropoietin (EPO) deposited on or embedded in a surface of the matrix. In one other embodiment, the EPO-expressing adipose stromal cell population is the KiSAS cell population described herein.

Brief Description of the Drawings

Figure 1A shows EPO expression by multiple cell types as determined by TaqMan qRTPCR comparative analysis of human cell and tissue sources. Figure 1B shows expression of EPO from kidney and non-kidney sourced adipose stromal cells is comparable to primary renal cells as determined by TaqMan qRT-PCR comparative analysis.

Figure 2 shows regulated expression of EPO (A.) and VEGF (B.) from rat visceral adipose stromal cells as determined by TaqMan qRT-PCR analysis.

Figure 3 shows regulated expression of EPO from human renal pedicle (A) and human major calyx (B) adipose stromal cells as determined by TaqMan qRT-PCR analysis, and regulated expression of VEGF from human renal pedicle (C) and human major calyx (D) adipose stromal cells as determined by TaqMan qRT-PCR analysis.

Figure 4A shows expression of EPO from human kidney and non-kidney sourced adipose stromal cells is comparable with primary renal cells, hepatocytes and keratinocytes. Figure 4B shows expression of EPO from canine kidney sourced adipose stromal (KiSAS) cells is comparable to canine primary renal cells.

Figure 5 shows the results of an analysis of EPO protein expression from adipose tissue by IEF.

Figure 6A shows semi-quantitative RT-PCR analysis of WT1 splice variants (KTS+/KTS-) in human kidney and non-kidney sourced adipose stromal cells. Figure 6B and C shows FACs analysis of the distribution of WT1+ cells in major calyx or renal pedicle associated adipose stromal cells. Figure 6D shows immuno-fluorescence analysis of the distribution of WT1+ cells in major calyx adipose stromal cells and renal pedicle adipose stromal cells.

Detailed Description of the Invention

The present invention concerns isolated cell populations derived from adipose tissue, methods of isolating such cell populations, scaffolds or matrices seeded with such cells (constructs) and methods of making the same, and methods of treating a patient in need using such cell populations or constructs. It has been discovered that cell populations derived from the stromal vascular fraction (SVF) of adipose tissue express erythropoietin (EPO). EPO expression may be expression of the EPO transcript and/or the EPO polypeptide. The cell populations also express VEGF transcript and/or VEGF polypeptide. The expression of EPO and/or VEGF is hypoxia-regulated expression. The adipose tissue may originate from any suitable source in the body including, without limitation, heart adipose, liver adipose, subcutaneous adipose, visceral adipose, white adipose, brown adipose.

In one embodiment, the adipose tissue may be renal adipose tissue. The renal adipose-derived cell population may be referred to herein as a kidney source adipose stromal (KiSAS) cell population. The KiSAS cell population expresses EPO and vascular endothelial growth factor (VEGF). The expression was found to occur in a hypoxia-regulated manner. Furthermore, it was discovered that the cells express the nephrogenic transcription factor WT1 that is specific to kidney adipose sourced stromal cells, and that niche specific adipose depots within kidney may be defined by distinctive WT1 transcriptional splice variants. The present invention demonstrates that adipose tissue is a functionally

unique depot for EPO-expressing cells that provides an alternate cell source for cell populations that may be used in tissue engineering and regenerative therapies of the kidney.

1. Definitions

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Principles of Tissue Engineering, 3rd Ed. (Edited by R Lanza, R Langer, & J Vacanti), 2007 provides one skilled in the art with a general guide to many of the terms used in the present application.

One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following terms are defined below.

The term "hypoxic" culture conditions as used herein refers to culture conditions in which cells are subjected to a reduction in available oxygen levels in the culture system relative to standard culture conditions in which cells are cultured at atmospheric oxygen levels (about 21%). Non-hypoxic conditions are referred to herein as normal or normoxic culture conditions.

The term "oxygen-tunable" as used herein refers to the ability of cells to modulate gene expression (up or down) based on the amount of oxygen available to the cells. "Hypoxia-inducible" refers to the upregulation of gene expression in response to a reduction in oxygen tension (regardless of the pre-induction or starting oxygen tension).

The term "construct" refers to one or more cell populations deposited on or in a surface of a scaffold or matrix made up of one or more synthetic or naturally-occurring biocompatible materials. The one or more cell populations may be coated with, deposited on, embedded in, attached to, seeded, or entrapped in a biomaterial made up of one or more synthetic or naturally-occurring biocompatible polymers, proteins, or peptides. The one or more cell populations may be combined with a biomaterial or scaffold or matrix *in vitro* or *in vivo*. In general, the one or more biocompatible materials used to form the scaffold/biomaterial is selected to direct, facilitate, or permit the formation of multi-cellular, three-dimensional, organization of at least one of the cell populations deposited thereon. The one or more biomaterials used to generate the construct may also be selected to direct, facilitate, or permit dispersion and/or integration of the construct or cellular components of the construct with the endogenous host tissue, or to direct, facilitate, or permit the survival, engraftment, tolerance, or functional performance of the construct or cellular components of the construct.

The term "marker" or "biomarker" refers generally to a DNA, RNA, protein, carbohydrate, or glycolipid-based molecular marker, the expression or presence of which in a cultured cell population can be detected by standard methods (or methods disclosed herein) and is consistent with one or more cells in the cultured cell population being a particular type of cell. The marker may be a polypeptide expressed by the cell or an identifiable physical location on a chromosome, such as a gene, a restriction endonuclease recognition site or a nucleic acid encoding a polypeptide (e.g., an mRNA) expressed by the

native cell. The marker may be an expressed region of a gene referred to as a "gene expression marker", or some segment of DNA with no known coding function. Biomarkers may be detected in cell populations directly derived from a tissue sample.

The terms "differentially expressed gene," "differential gene expression" and their synonyms, which are used interchangeably, refer to a gene whose expression is activated to a higher or lower level in a first cell or cell population, relative to its expression in a second cell or cell population. The terms also include genes whose expression is activated to a higher or lower level at different stages over time during passage of the first or second cell in culture. It is also understood that a differentially expressed gene may be either activated or inhibited at the nucleic acid level or protein level, or may be subject to alternative splicing to result in a different polypeptide product. Such differences may be evidenced by a change in mRNA levels, surface expression, secretion or other partitioning of a polypeptide, for example. Differential gene expression may include a comparison of expression of one or more genes or their gene products, or a comparison of the ratios of the expression between one or more genes or their gene products, or even a comparison of two differently processed products of the same gene, which differ between the first cell/cell population and the second cell/cell population. Differential expression includes both quantitative, as well as qualitative, differences in the temporal or cellular expression pattern in a gene or its expression products among, for example, a first cell population and a second cell population. For the purpose of this invention, "differential gene expression" is considered to be present when there is a difference between the expression of a given gene in the first cell population and the second cell population.

The terms "inhibit", "down-regulate", "under-express" and "reduce" are used interchangeably and mean that the expression of a gene, or level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits, is reduced relative to one or more controls, such as, for example, one or more positive and/or negative controls.

The term "up-regulate" or "over-express" is used to mean that the expression of a gene, or level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits, is elevated relative to one or more controls, such as, for example, one or more positive and/or negative controls.

The term "subject" shall mean any single human subject, including a patient, eligible for treatment, who is experiencing or has experienced one or more signs, symptoms, or other indicators of a kidney disease, anemia, or EPO deficiency. Such subjects include without limitation subjects who are newly diagnosed or previously diagnosed and are now experiencing a recurrence or relapse, or are at risk for a kidney disease, anemia, or EPO deficiency, no matter the cause. The subject may have been previously treated for a kidney disease, anemia, or EPO deficiency, or not so treated.

The term "patient" refers to any single animal, more preferably a mammal (including such non-human animals as, for example, dogs, cats, horses, rabbits, zoo animals, cows, pigs, sheep, and non-human primates) for which treatment is desired. Most preferably, the patient herein is a human.

The term "sample" or "patient sample" or "biological sample" shall generally mean any biological sample obtained from a subject or patient, body fluid, body tissue, cell line, tissue culture, or other source. The term includes tissue biopsies such as, for example, kidney biopsies. The term includes cultured cells such as, for example, cultured mammalian kidney cells. Methods for obtaining tissue biopsies and cultured cells from mammals are well known in the art. If the term "sample" is used alone, it shall still mean that the "sample" is a "biological sample" or "patient sample", *i.e.,* the terms are used interchangeably.

The term "anemia" as used herein refers to a deficit in red blood cell number and/or hemoglobin levels due to inadequate production of functional EPO protein by the EPO-producing cells of a subject, and/or inadequate release of EPO protein into systemic circulation, and/or the inability of erythroblasts in the bone marrow to respond to EPO protein. A subject with anemia is unable to maintain erythroid homeostasis. In general, anemia can occur with a decline or loss of kidney function (e.g., chronic renal failure), anemia associated with relative EPO deficiency, anemia associated with congestive heart failure, anemia associated with myelo-suppressive therapy such as chemotherapy or anti-viral therapy (e.g., AZT), anemia associated with non-myeloid cancers, anemia associated with viral infections such as HIV, and anemia of chronic diseases such as autoimmune diseases (e.g., rheumatoid arthritis), liver disease, and multi-organ system failure.

The term "EPO-deficiency" refers to any condition or disorder that is treatable with an erythropoietin receptor agonist (e.g., recombinant EPO, EPO peptide mimetics, or EPO analogs) or erythropoiesis-stimulating agent (ESA), including anemia.

The term "kidney disease" as used herein refers to disorders associated with any stage or degree of acute or chronic renal failure that results in a loss of the kidney's ability to perform the function of blood filtration and elimination of excess fluid, electrolytes, and wastes from the blood. Kidney disease also includes endocrine dysfunctions such as anemia (erythropoietin-deficiency), and mineral imbalance (Vitamin D deficiency). Kidney disease may originate in the kidney or may be secondary to a variety of conditions, including (but not limited to) heart failure, hypertension, diabetes, autoimmune disease, or liver disease. Kidney disease may be a condition of chronic renal failure that develops after an acute injury to the kidney. For example, injury to the kidney by ischemia and/or exposure to toxicants may cause acute renal failure; incomplete recovery after acute kidney injury may lead to the development of chronic renal failure.

The term "treatment" refers to both therapeutic treatment and prophylactic or preventative measures for kidney disease, anemia, EPO deficiency, tubular transport deficiency, or glomerular filtration deficiency wherein the object is to reverse, prevent or slow down (lessen) the targeted disorder. Those in need of treatment include those already having a kidney disease, anemia, EPO deficiency, tubular transport deficiency, or glomerular filtration deficiency as well as those prone to having a kidney disease, anemia, EPO deficiency, tubular transport deficiency, or glomerular filtration deficiency or those in whom the kidney disease, anemia, EPO deficiency, tubular transport deficiency, or glomerular

filtration deficiency is to be prevented. The term "treatment" as used herein includes the stabilization and/or improvement of kidney function.

2. Cell populations

The present invention provides populations of adipose tissue-derived cells that express erythropoietin (EPO) as shown by expression of the EPO transcript and/or the EPO polypeptide. The cells also express the VEGF transcript and/or the VEGF polypeptide. EPO and VEGF expression are hypoxia-regulated. The adipose tissue may originate from any suitable source in the body including, without limitation, heart adipose, liver adipose, subcutaneous adipose, visceral adipose, white adipose, brown adipose. In one embodiment, the cell populations described herein may be derived from an adipose source autologous to the subject in need of treatment. The cell populations may also be derived from adipose sources that are non-autologous to the subject including, without limitation, allogeneic, or syngeneic (autogeneic or isogeneic) sources.

In one embodiment, the adipose tissue source may be renal adipose tissue. The cell populations derived from renal adipose may be referred to as kidney sourced adipose stromal (KiSAS) cell populations. The KiSAS cell populations may be derived from any adipose tissue associated with the kidney including, without limitation, adipose tissue associated with the renal pedicle and/or renal calyx (major or minor). In one embodiment, the KiSAS cell population is derived from a renal adipose stromal vascular fraction (SVF).

In one aspect, the adipose-derived cell populations express EPO in a manner that is regulated by hypoxic conditions. The cell populations of the present invention are characterized by EPO expression and bioresponsiveness to oxygen, such that a reduction in the oxygen tension of the culture system results in an induction in the expression of EPO. In one embodiment, the EPO expression is induced when the cell population is cultured under conditions where the cells are subjected to a reduction in available oxygen levels in the culture system as compared to a cell population cultured at normal atmospheric (~21%) levels of available oxygen. In one embodiment, the cells cultured in lower oxygen conditions express greater levels of EPO relative to cells cultured at normal oxygen conditions. In general, the culturing of cells at reduced levels of available oxygen (also referred to as hypoxic culture conditions) means that the level of reduced oxygen is reduced relative to the culturing of cells at normal atmospheric levels of available oxygen (also referred to as normal or normoxic culture conditions). In one embodiment, hypoxic cell culture conditions include culturing cells at about less than 1% oxygen, about less than 2% oxygen, about less than 3% oxygen, about less than 4% oxygen, or about less than 5% oxygen. In another embodiment, normal or normoxic culture conditions include culturing cells at about 10% oxygen, about 12% oxygen, about 13% oxygen, about 14% oxygen, about 15% oxygen, about 16% oxygen, about 17% oxygen, about 18% oxygen, about 19% oxygen, about 20% oxygen, or about 21% oxygen.

In one other embodiment, the induction or increased expression of EPO is obtained and can be observed by culturing cells at about less than 5% available oxygen and comparing EPO expression levels

to cells cultured at atmospheric (about 21%) oxygen. In another embodiment, the induction of EPO is obtained in a culture of cells capable of expressing EPO by a method that includes a first culture phase in which the culture of cells is cultivated at atmospheric oxygen (about 21%) for some period of time and a second culture phase in which the available oxygen levels are reduced and the same cells are cultured at about less than 5% available oxygen.

In another embodiment, the EPO expression that is responsive to hypoxic conditions is regulated by HIF1α. Those of ordinary skill in the art will appreciate that other oxygen manipulation culture conditions known in the art may be used for the cells described herein.

In one aspect, the cell populations described herein differ in their expression of EPO, as compared to other cell populations and/or adipose sources known to express EPO. The difference may be at the transcript level, the polypeptide level, and/or the post-translational level.

In one embodiment, the KiSAS cell population expresses EPO polypeptide at a higher level as compared to non-adipose cell populations. The non-adipose cell populations include, without limitation, liver adipose, heart adipose, white adipose, and brown adipose. This is illustrated in Figure 5 (see Example 1).

It has been discovered that the adipose-derived EPO-expressing cell populations of the present invention express EPO with specific post-translation modifications when compared to the EPO expressed by other cell populations. This can be demonstrated through the use of various techniques. For example, isoelectric focusing (IEF) has been used to detect different forms of EPO in samples obtained from individuals (Catlin et al. Clinical Chemistry 48;11, 2057-2059 (2002); Breidbach et al. Clinical Chemistry 49:6, 901-907 (2003)). As described in Example 1, the EPO from renal adipose tissue subjected to IEF was found to have a different migration pattern when compared to other potential sources of EPO including keratinocytes, hepatocytes, non-renal adipose, and primary kidney cell populations (Figure 4A). Lane 3 corresponds to EPO expressed in a KiSAS cell population and the migration pattern is different from the other lanes. For example, if the IEF gel has a pH gradient where the top of the gel is the most acidic (+) and the bottom of the gel is the most basic (-), then the KiSAS EPO migrates to a point more acidic than the EPO from a visceral (non-renal) adipose stromal cell population (lane 4). In one embodiment, the KiSAS cell population expresses EPO that is more acidic (as shown by IEF) when compared to the EPO expressed by a cell population selected from the group consisting of keratinocytes, hepatocytes, visceral (non-renal) adipose stromal cell, and primary kidney cell populations.

Figure 4A also shows that EPO expressed in visceral adipose stromal cells migrated had a different migration pattern when compared to EPO from keratinocytes, hepatocytes, renal adipose, and primary kidney cell populations. Lane 4 corresponds to EPO expressed in a visceral adipose cell population and the migration pattern is different from the other lanes. For example, the visceral adipose EPO migrates to a point less acidic than the EPO from a renal adipose stromal cell population (lane 3) or EPO from primary kidney cell populations (lanes 6-7). In one embodiment, the visceral adipose cell population expresses EPO that is less acidic (as shown by IEF) when compared to the EPO expressed by

a cell population selected from the group consisting of keratinocytes, hepatocytes, renal adipose stromal cell, and primary kidney cell populations.

In another aspect, the KiSAS cell population expresses biomarkers associated with regeneration. In one embodiment, the biomarker is WT-1. The KiSAS cell population may express one or more such biomarkers at a higher level than a non-renal sourced adipose stromal cell population. The non-renal sourced adipose stromal cell population may be a visceral adipose stromal cell population.

3. Methods of isolating EPO-expressing cell populations

It has unexpectedly been discovered that adipose tissue is a source of cells that express EPO. The present invention, in one aspect, provides methods for separating and isolating adipose cell populations that express erythropoietin (EPO), for therapeutic use, including the treatment of kidney disease, anemia, EPO deficiency, tubular transport deficiency, and glomerular filtration deficiency.

Autologous kidney sourced adipose stromal cell populations may be derived directly from the subjects in need of treatment. Non-autologous cell populations may be derived from suitable donors. The cells may be isolated in biopsies. In addition, the cells may be frozen or expanded before use.

For renal adipose, the cell populations are isolated from freshly digested, i.e., mechanically or enzymatically digested, adipose kidney tissue or from in vitro cultures of mammalian kidney cells. Renal adipose tissue can be obtained from the renal pedicle or major calyx. The pedicle may be dissected away from the kidney. Renal adipose tissue from the major calyx can be dissected away from the medulla in a bisection of a whole human kidney. The calyx adipose may be dissected away from the medulla.

Following dissection, the adipose tissue samples can be processed according to the following exemplary protocol (see Example 1). Adipose tissue is extensively washed with PBS/0.1% gentamicin (Invitrogen-Gibco) and digested for up to 1 hour with 0.3% collagenase I (Worthington), 1% BSA in DMEM-HG (Invitrogen-Gibco) at 37° C. Samples can be centrifuged at 600g for 20 minutes and the adipocytic supernatant aspirated away. The remaining stromal vascular fraction can then be re-suspended in α-MEM/10% FBS (Invitrogen-Gibco) and placed in a tissue culture incubator for 24-48 hours. Non-adherent cell populations may be removed by washing 3X with PBS. The cell populations may be cultured under hypoxic conditions by maintaining them in an O_2-depleted (hypoxic) (2%) incubator for various time periods. To confirm the integrity of hypoxic regulation pathways, the cell populations may be checked for expression of biomarkers, *e.g.*, EPO and/or VEGF.

In one aspect, the cell populations of the present invention are characterized by EPO expression and bioresponsiveness to oxygen, such that a reduction in the oxygen tension of the culture system results in an induction in the expression of EPO. The cell populations are oxygen-tunable. In one embodiment, the EPO expression is induced when the cell population is cultured under conditions where the cells are subjected to a reduction in available oxygen levels in the culture system as compared to a cell population cultured at normal atmospheric (~21%) levels of available oxygen. In one embodiment, the cells cultured in lower oxygen conditions express greater levels of EPO relative to EPO-producing cells cultured at normal oxygen conditions. In general, the culturing of cells at reduced levels of available oxygen (also

referred to as hypoxic culture conditions) means that the level of reduced oxygen is reduced relative to the culturing of cells at normal atmospheric levels of available oxygen (also referred to as normal or normoxic culture conditions). In one embodiment, hypoxic cell culture conditions include culturing cells at about less than 1% oxygen, about less than 2% oxygen, about less than 3% oxygen, about less than 4% oxygen, or about less than 5% oxygen. In another embodiment, normal or normoxic culture conditions include culturing cells at about 10% oxygen, about 12% oxygen, about 13% oxygen, about 14% oxygen, about 15% oxygen, about 16% oxygen, about 17% oxygen, about 18% oxygen, about 19% oxygen, about 20% oxygen, or about 21% oxygen.

In one other embodiment, the induction or increased expression of EPO is obtained and can be observed by culturing cells at about less than 5% available oxygen and comparing EPO expression levels to cells cultured at atmospheric (about 21%) oxygen. In another embodiment, the induction of EPO is obtained in a culture of cells capable of expressing EPO by a method that includes a first culture phase in which the culture of cells is cultivated at atmospheric oxygen (about 21%) for some period of time and a second culture phase in which the available oxygen levels are reduced and the same cells are cultured at about less than 5% available oxygen. In another embodiment, the EPO expression that is responsive to hypoxic conditions is regulated by HIF1α. Those of ordinary skill in the art will appreciate that other oxygen manipulation culture conditions known in the art may be used for the cells described herein.

Exemplary techniques for separating and isolating the cell populations of the invention include separation on a density gradient based on the differential specific gravity of different cell types contained within the population of interest. The specific gravity of any given cell type can be influenced by the degree of granularity within the cells, the intracellular volume of water, and other factors. In one aspect, the present invention provides optimal gradient conditions for isolation of the cell populations across multiple species including, but not limited to, human, canine, and rodent.

In another aspect, the present invention provides methods of enriching and/or depleting cell types using fluorescent activated cell sorting (FACS). In one embodiment, cell types may be enriched and/or depleted using BD FACSAria™ or equivalent.

In another aspect, the present invention provides methods of enriching and/or depleting cell types using magnetic cell sorting. In one embodiment, the KiSAS cell population may be enriched and/or depleted using the Miltenyi autoMACS® system or equivalent.

In another aspect, the present invention provides methods of three-dimensional culturing of the adipose-derived cell populations. In one aspect, the present invention provides methods of culturing the cell populations via continuous perfusion. In one embodiment, the perfusion conditions include transient, intermittent, or continuous fluid flow (perfusion). In one embodiment, the media in which the cells are cultured is intermittently or continuously circulated or agitated in such a manner that dynamic forces are transferred to the cells via the flow. In one embodiment, the cells subjected to the transient, intermittent, or continuous fluid flow are cultured in such a manner that they are present as three-dimensional structures in or on a material that provides framework and/or space for such three-dimensional structures to form. In one embodiment, the cells are cultured on porous beads and subjected to intermittent or

continuous fluid flow by means of a rocking platform, orbiting platform, or spinner flask. In another embodiment, the cells are cultured on three-dimensional scaffolding and placed into a device whereby the scaffold is stationary and fluid flows directionally through or across the scaffolding. Those of ordinary skill in the art will appreciate that other perfusion culture conditions known in the art may be used for the cells described herein.

As described herein, low or hypoxic oxygen conditions may be used in the methods to prepare the cell populations of the present invention. However, the methods of the present invention may be used without the step of low oxygen conditioning. In one embodiment, normoxic conditions may be used.

Those of ordinary skill in the art will appreciate that other methods of isolation and culturing known in the art may be used for the cells described herein.

4. Matrices or scaffolds

As described in Bertram et al. U.S. Published Application 20070276507 (incorporated herein by reference in its entirety), polymeric matrices or scaffolds may be shaped into any number of desirable configurations to satisfy any number of overall system, geometry or space restrictions. In one embodiment, the matrices or scaffolds of the present invention may be three-dimensional and shaped to conform to the dimensions and shapes of an organ or tissue structure. For example, in the use of the polymeric scaffold for treating kidney disease, anemia, EPO deficiency, tubular transport deficiency, or glomerular filtration deficiency, a three-dimensional (3-D) matrix may be used. A variety of differently shaped 3-D scaffolds may be used. Naturally, the polymeric matrix may be shaped in different sizes and shapes to conform to differently sized patients. The polymeric matrix may also be shaped in other ways to accommodate the special needs of the patient. In another embodiment, the polymeric matrix or scaffold may be a biocompatible, porous polymeric scaffold. The scaffolds may be formed from a variety of synthetic or naturally-occurring materials including, but not limited to, open-cell polylactic acid (OPLA®), cellulose ether, cellulose, cellulosic ester, fluorinated polyethylene, phenolic, poly-4-methylpentene, polyacrylonitrile, polyamide, polyamideimide, polyacrylate, polybenzoxazole, polycarbonate, polycyanoarylether, polyester, polyestercarbonate, polyether, polyetheretherketone, polyetherimide, polyetherketone, polyethersulfone, polyethylene, polyfluoroolefin, polyimide, polyolefin, polyoxadiazole, polyphenylene oxide, polyphenylene sulfide, polypropylene, polystyrene, polysulfide, polysulfone, polytetrafluoroethylene, polythioether, polytriazole, polyurethane, polyvinyl, polyvinylidene fluoride, regenerated cellulose, silicone, urea-formaldehyde, collagens, laminins, fibronectin, silk, elastin, alginate, hyaluronic acid, agarose, or copolymers or physical blends thereof. Scaffolding configurations may range from liquid hydrogel suspensions to soft porous scaffolds to rigid, shape-holding porous scaffolds.

Hydrogels may be formed from a variety of polymeric materials and are useful in a variety of biomedical applications. Hydrogels can be described physically as three-dimensional networks of hydrophilic polymers. Depending on the type of hydrogel, they contain varying percentages of water, but altogether do not dissolve in water. Despite their high water content, hydrogels are capable of

additionally binding great volumes of liquid due to the presence of hydrophilic residues. Hydrogels swell extensively without changing their gelatinous structure. The basic physical features of hydrogel can be specifically modified, according to the properties of the polymers used and the additional special equipments of the products.

Preferably, the hydrogel is made of a polymer, a biologically derived material, a synthetically derived material or combinations thereof, that is biologically inert and physiologically compatible with mammalian tissues. The hydrogel material preferably does not induce an inflammatory response. Examples of other materials which can be used to form a hydrogel include (a) modified alginates, (b) polysaccharides (e.g. gellan gum and carrageenans) which gel by exposure to monovalent cations, (c) polysaccharides (e.g., hyaluronic acid) that are very viscous liquids or are thixotropic and form a gel over time by the slow evolution of structure, and (d) polymeric hydrogel precursors (e.g., polyethylene oxide-polypropylene glycol block copolymers and proteins). U.S. Pat. No. 6,224,893 B1 provides a detailed description of the various polymers, and the chemical properties of such polymers, that are suitable for making hydrogels in accordance with the present invention.

Scaffolding or biomaterial characteristics may enable cells to attach and interact with the scaffolding or biomaterial material, and/or may provide porous spaces into which cells can be entrapped. In one embodiment, the porous scaffolds or biomaterials of the present invention allow for the addition or deposition of a population of cells on a biomaterial configured as a porous scaffold (e.g., by attachment of the cells) and/or within the pores of the scaffold (e.g., by entrapment of the cells). In another embodiment, the scaffolds or biomaterials allow or promote for cell:cell and/or cell:biomaterial interactions within the scaffold to form constructs as described herein.

In one embodiment, the biomaterial used in accordance with the present invention is comprised of hyaluronic acid (HA) in hydrogel form, containing HA molecules ranging in size from 5.1 kDA to >2 x 10^6 kDa. In another embodiment, the biomaterial used in accordance with the present invention is comprised of hyaluronic acid in porous foam form, also containing HA molecules ranging in size from 5.1 kDA to >2 x 10^6 kDa . In yet another embodiment, the biomaterial used in accordance with the present invention is comprised of a poly-lactic acid (PLA)-based foam, having an open-cell structure and pore size of about 50 microns to about 300 microns.

Those of ordinary skill in the art will appreciate that other types of synthetic or naturally-occurring materials known in the art may be used to form scaffolds as described herein.

5. Constructs

In one aspect, the invention provides one or more polymeric scaffolds or matrices that are seeded or deposited with an adipose-derived EPO-expressing cell population described herein. Such scaffolds that have been seeded with a cell population and may be referred to herein as "constructs".

In one aspect, the invention provides implantable constructs having one or more of the cell populations described herein for the treatment of kidney disease, anemia, or EPO deficiency in a subject in need. In one embodiment, the construct is made up of a biocompatible material or biomaterial,

scaffold or matrix composed of one or more synthetic or naturally-occurring biocompatible materials and a cell population described herein deposited on or embedded in a surface of the scaffold by attachment and/or entrapment. In certain embodiments, the construct is made up of a biomaterial and a cell population coated with, deposited on, deposited in, attached to, entrapped in, embedded in, or combined with the biomaterial component(s). The cell population may be used in combination with a matrix to form a construct. In one embodiment, the cell population is directly contacted with the matrix to form a construct.

In another embodiment, the deposited cell population or cellular component of the construct is a KiSAS cell population which expresses EPO. The seeded KiSAS cell population may be oxygen-tunable EPO-expressing cells. In other embodiments, the KiSAS cells are characterized by the expression of one or more biomarkers. The biomarker may be a tubular cell biomarker selected from the group consisting of cubilin; E-cadherin; NPHS1 (nephrosis 1, congenital, Finnish type (nephrin)); podocalyxin; Vitamin D3 25-Hydroxylase (CYP2D25), Wnt4, and any combination thereof. In another embodiment, the biomarker may be selected from the group consisting of WT1, VEGF, EPO, and any combination thereof. The biomarker may be a renal regenerative biomarker known to be involved in renal organ development, *e.g.*, WT1.

In one embodiment, the construct deposited with cells is adapted to improve kidney function in a subject upon implantation into or administration to the subject' kidney.

In one embodiment, the cell populations deposited on or combined with biomaterials or scaffolds to form constructs of the present invention are derived from a variety of sources, such as autologous sources. Non-autologous sources are also suitable for use, including without limitation, allogeneic, or syngeneic (autogeneic or isogeneic) sources.

Those of ordinary skill in the art will appreciate there are several suitable methods for depositing or otherwise combining cell populations with biomaterials to form a construct.

6. Methods of use

In one aspect, the present invention contemplates methods for providing an adipose-derived cell population or construct to a subject in need of such treatment. In one embodiment, the method includes the step of providing a matrix that will be seeded or deposited with a cell population to form a construct. The depositing step may include culturing the cell population on the matrix. After depositing the cell population on the matrix to form a construct, it can be implanted into a patient at the site of treatment.

In one aspect, the present invention provides methods for the treatment of a kidney disease, anemia, or EPO deficiency in a subject in need with the cell populations described herein. In one embodiment, the method comprises administering to the subject a composition that includes a cell population. In another embodiment, the composition also includes a cell population deposited in, deposited on, embedded in, coated with, or entrapped in a biomaterial to form an implantable construct, as described herein, for the treatment of a disease or disorder described herein. In one embodiment, the cell populations are used alone or in combination with other cells or biomaterials, e.g., hydrogels, porous

scaffolds, or native or synthetic peptides or proteins, to stimulate regeneration in acute or chronic disease states.

In another aspect, the effective treatment of a kidney disease, anemia, or EPO deficiency in a subject by the methods of the present invention can be observed through various indicators of erythropoiesis and/or kidney function. In one embodiment, the indicators of erythroid homeostasis include, without limitation, hematocrit (HCT), hemoglobin (HB), mean corpuscular hemoglobin (MCH), red blood cell count (RBC), reticulocyte number, reticulocyte %, mean corpuscular volume (MCV), and red blood cell distribution width (RDW). In one other embodiment, the indicators of kidney function include, without limitation, serum albumin, albumin to globulin ratio (A/G ratio), serum phosphorous, serum sodium, kidney size (measurable by ultrasound), serum calcium, phosphorous:calcium ratio, serum potassium, proteinuria, urine creatinine, serum creatinine, blood nitrogen urea (BUN), cholesterol levels, triglyceride levels and glomerular filtration rate (GFR). Furthermore, several indicators of general health and well-being include, without limitation, weight gain or loss, survival, blood pressure (mean systemic blood pressure, diastolic blood pressure, or systolic blood pressure), and physical endurance performance.

In another embodiment, an effective treatment is evidenced by stabilization of one or more indicators of kidney function. The stabilization of kidney function is demonstrated by the observation of a change in an indicator in a subject treated by a method of the present invention as compared to the same indicator in a subject that has not been treated by a method of the present invention. Alternatively, the stabilization of kidney function may be demonstrated by the observation of a change in an indicator in a subject treated by a method of the present invention as compared to the same indicator in the same subject prior to treatment. The change in the first indicator may be an increase or a decrease in value. In one embodiment, the treatment provided by the present invention may include stabilization of blood urea nitrogen (BUN) levels in a subject where the BUN levels observed in the subject are lower as compared to a subject with a similar disease state who has not been treated by the methods of the present invention. In one other embodiment, the treatment may include stabilization of serum creatinine levels in a subject where the serum creatinine levels observed in the subject are lower as compared to a subject with a similar disease state who has not been treated by the methods of the present invention. In another embodiment, the treatment may include stabilization of hematocrit (HCT) levels in a subject where the HCT levels observed in the subject are higher as compared to a subject with a similar disease state who has not been treated by the methods of the present invention. In another embodiment, the treatment may include stabilization of red blood cell (RBC) levels in a subject where the RBC levels observed in the subject are higher as compared to a subject with a similar disease state who has not been treated by the methods of the present invention. Those of ordinary skill in the art will appreciate that one or more additional indicators described herein or known in the art may be measured to determine the effective treatment of a kidney disease in the subject.

In another aspect, the present invention concerns a method of providing erythroid homeostasis in a subject in need. In one embodiment, the method includes the step of (a) administering to the subject a cell population; and (b) determining, in a biological sample from the subject, that the level of an

erythropoiesis indicator is different relative to the indicator level in a control, wherein the difference in indicator level (i) indicates the subject is responsive to the administering step (a), or (ii) is indicative of erythroid homeostasis in the subject. In another embodiment, the method includes the step of (a) administering to the subject a composition comprising a cell population as described herein; and (b) determining, in a biological sample from the subject, that the level of an erythropoiesis indicator is different relative to the indicator level in a control, wherein the difference in indicator level (i) indicates the subject is responsive to the administering step (s), or (ii) is indicative of erythroid homeostasis in the subject. In another embodiment, the method includes the step of (a) providing a biomaterial or biocompatible polymeric scaffold; (b) depositing a cell population of the present invention on or within the biomaterial or scaffold in a manner described herein to form an implantable construct; (c) implanting the construct into the subject; and (d) determining, in a biological sample from the subject, that the level of an erythropoiesis indicator is different relative to the indicator level in a control, wherein the difference in indicator level (i) indicates the subject is responsive to the administering step (a), or (ii) is indicative of erythroid homeostasis in the subject.

In another aspect, the present invention concerns a method of providing both stabilization of kidney function and restoration of erythroid homeostasis to a subject in need, said subject having both a deficit in kidney function and an anemia and/or EPO-deficiency. In one embodiment, the method includes the step of administering a cell population or a construct containing the same. In this embodiment, treatment of the subject would be demonstrated by an improvement in at least one indicator of kidney function concomitant with improvement in at least one indicator of erythropoiesis, compared to either an untreated subject or to the subject's pre-treatment indicators.

In one aspect, the present invention provides methods of (i) treating a kidney disease, anemia, or an EPO-deficiency; (ii) stabilizing kidney function, (iii) restoring erythroid homeostasis, or (iv) any combination of thereof by administering an adipose-derived EPO-expressing cell population, wherein the beneficial effects of the administration are greater than, or an improvement over, the effects of administering a cell population that is not an adipose-derived EPO-expressing cell population, or greater than, or an improvement over, the effect of not administering the adipose-derived cell population. In another embodiment, the adipose cell population provides an improved level of serum blood urea nitrogen (BUN). In another embodiment, the adipose cell population provides an improved retention of protein in the serum. In another embodiment, the adipose cell population provides improved levels of serum cholesterol and/or triglycerides. In another embodiment, the adipose cell population provides an improved level of Vitamin D. In one embodiment, the adipose cell population provides an improved phosphorus:calcium ratio. In another embodiment, the adipose cell population provides an improved level of hemoglobin. In a further embodiment, the adipose cell population provides an improved level of serum creatinine. In yet another embodiment, the adipose cell population provides an improved level of hematocrit. In a further embodiment, the adipose cell population provides an improved level of red blood cell number (RBC#). In one embodiment, the improved level of hematocrit is restored to 95% normal healthy level. In a further embodiment, the a adipose cell population provides an improved reticulocyte

number. In other embodiments, the adipose cell population provides an improved reticulocyte percentage. In yet other embodiments, the adipose cell population provides an improved level of red blood cell volume distribution width (RDW). In yet another embodiment, the adipose cell population provides an improved level of hemoglobin. In yet another embodiment, the adipose cell population provides an erythropoietic response in the bone marrow, such that the marrow cellularity is near-normal and the myeloid:erythroid ratio is near normal. In one other embodiment, the adipose cell population provides improved blood pressure.

In another aspect, the present invention provides methods of (i) treating a kidney disease, anemia, or an EPO-deficiency; (ii) stabilizing kidney function, (iii) restoring erythroid homeostasis, or (iv) any combination of thereof by administering an adipose-derived EPO-expressing cell population, wherein the beneficial effects of administering an adipose cell population described herein are characterized by comparable or improved erythroid homeostasis when compared to the beneficial effects provided by the administering of recombinant EPO (rEPO).

In one embodiment, the adipose-derived EPO-expressing cell population, when administered to a subject in need provides improved erythroid homeostasis (as determined by hematocrit, hemoglobin, or RBC#) when compared to the administration of recombinant EPO protein. In one embodiment, the adipose cell population, when administered provides an improved level of hematocrit, RBC, or hemoglobin as compared to recombinant EPO. In a further embodiment, a single dose or delivery of the adipose cell population, when administered provides improvement in erythroid homeostasis (as determined by increase in hematocrit, hemoglobin, or RBC#) in the treated subject for a period of time that significantly exceeds the period of time that a single dose or delivery of the recombinant EPO protein provides improvement in erythroid homeostasis. In another embodiment, the recombinant EPO is delivered at a dose of about 100 IU/kg, about 200 IU/kg, about 300 IU/kg, about 400 IU/kg, or about 500 IU/kg. Those of ordinary skill in the art will appreciate that other dosages of recombinant EPO known in the art may be suitable.

Another embodiment of the present invention is directed to the use of an adipose-derived EPO-expressing cell population, described herein, or an implantable construct described herein, for the preparation of a medicament useful in the treatment of a kidney disease, anemia, or EPO deficiency in a subject in need, the providing of erythroid homeostasis in a subject in need, or the improvement of kidney function in a subject in need.

In yet another aspect, the present invention provides a method of treating a kidney disease in a subject in need, comprising: administering to the subject a composition comprising an adipose-derived EPO-expressing cell population or a construct comprising the same. In certain embodiments, the method includes determining in a test sample from the subject that the level of a kidney function indicator is different relative to the indicator level in a control, wherein the difference in indicator level is indicative of a reduction in decline, stabilization, or an improvement of one or more kidney functions in the subject.

In certain embodiments, the kidney disease to be treated by the methods of the invention is accompanied by an erythropoietin (EPO) deficiency. In certain embodiments, the EPO deficiency is

anemia. In some embodiments, the EPO deficiency or anemia occurs secondary to renal failure in the subject. In some other embodiments, the EPO deficiency or anemia occurs secondary to a disorder selected from the group consisting of chronic renal failure, primary EPO deficiency, chemotherapy or anti-viral therapy, non-myeloid cancer, HIV infection, liver disease, cardiac failure, rheumatoid arthritis, or multi-organ system failure. In certain embodiments, the composition used in the method further comprises a biomaterial comprising one or more biocompatible synthetic polymers and/or naturally-occurring proteins or peptides, wherein the adipose cell population is coated with, deposited on or in, entrapped in, suspended in, embedded in and/or otherwise combined with the biomaterial. In certain embodiments, the adipose cell population used in the methods of the invention is derived from mammalian adipose tissue or cultured adipose tissue cells. In other embodiments, the adipose cell population is derived from a kidney sample that is autologous to the subject in need. In one embodiment, the sample is a renal adipose tissue biopsy. In other embodiments, the adipose cell population used in the methods of the invention is derived from a non-autologous renal adipose tissue sample.

In yet another aspect, the invention provides the use of the adipose cell populations or an implantable construct of the instant invention for the preparation of a medicament useful in the treatment of a kidney disease, anemia or EPO deficiency in a subject in need thereof.

In another aspect, the present invention provides methods for the regeneration of renal function in a native kidney in a subject in need thereof. In one embodiment, the method includes the step of administering or implanting an adipose cell population or construct described herein to the subject's kidney. A regenerated renal function in a native kidney may be characterized by a number of indicators including, without limitation, development of function or capacity in the native kidney, improvement of function or capacity in the native kidney, and the expression of certain markers in the native kidney. In one embodiment, the developed or improved function or capacity may be observed based on the various indicators of erythroid homeostasis and kidney function described above.

The adipose cell populations described herein, as well as constructs containing the same, may be used to provide a regenerative effect to a native kidney. The regenerative effect may be characterized by stabilization of one or more indicators of kidney function (as described herein) and/or restoration of erythroid homeostasis (as described herein).

7. Methods and Routes of Administration

The adipose cell populations and/or constructs of the instant invention can be administered alone or in combination with other bioactive components.

The therapeutically effective amount of the adipose cell populations described herein can range from the maximum number of cells that is safely received by the subject to the minimum number of cells necessary for treatment of kidney disease, *e.g.,* stabilization, reduced rate-of-decline, or improvement of one or more kidney functions. In certain embodiments, the methods of the present invention provide the administration of adipose cell populations described herein at a dosage of about 10,000 cells/kg, about 20,000 cells/kg, about 30,000 cells/kg, about 40,000 cells/kg, about 50,000 cells/kg, about 100,000

cells/kg, about 200,000 cells/kg, about 300,000 cells/kg, about 400,000 cells/kg, about 500,000 cells/kg, about 600,000 cells/kg, about 700,000 cells/kg, about 800,000 cells/kg, about 900,000 cells/kg, about $1.1x10^6$ cells/kg, about $1.2x10^6$ cells/kg, about $1.3x10^6$ cells/kg, about $1.4x10^6$ cells/kg, about $1.5x10^6$ cells/kg, about $1.6x10^6$ cells/kg, about $1.7x10^6$ cells/kg, about $1.8x10^6$ cells/kg, about $1.9x10^6$ cells/kg, about $2.1x10^6$ cells/kg, about $2.1x10^6$ cells/kg, about $1.2x10^6$ cells/kg, about $2.3x10^6$ cells/kg, about $2.4x10^6$ cells/kg, about $2.5x10^6$ cells/kg, about $2.6x10^6$ cells/kg, about $2.7x10^6$ cells/kg, about $2.8x10^6$ cells/kg, about $2.9x10^6$ cells/kg, about $3x10^6$ cells/kg, about $3.1x10^6$ cells/kg, about $3.2x10^6$ cells/kg, about $3.3x10^6$ cells/kg, about $3.4x10^6$ cells/kg, about $3.5x10^6$ cells/kg, about $3.6x10^6$ cells/kg, about $3.7x10^6$ cells/kg, about $3.8x10^6$ cells/kg, about $3.9x10^6$ cells/kg, about $4x10^6$ cells/kg, about $4.1x10^6$ cells/kg, about $4.2x10^6$ cells/kg, about $4.3x10^6$ cells/kg, about $4.4x10^6$ cells/kg, about $4.5x10^6$ cells/kg, about $4.6x10^6$ cells/kg, about $4.7x10^6$ cells/kg, about $4.8x10^6$ cells/kg, about $4.9x10^6$ cells/kg, or about $5x10^6$ cells/kg. In another embodiment, the dosage of cells to a subject may be a single dosage or a single dosage plus additional dosages. In other embodiments, the dosages may be provided by way of a construct as described herein. In other embodiments, the dosage of cells to a subject may be calculated based on the estimated renal mass or functional renal mass.

The therapeutically effective amount of the cell populations or admixtures thereof described herein can be suspended in a pharmaceutically acceptable carrier or excipient. Such a carrier includes, but is not limited to basal culture medium plus 1% serum albumin, saline, buffered saline, dextrose, water, collagen, alginate, hyaluronic acid, fibrin glue, polyethyleneglycol, polyvinylalcohol, carboxymethylcellulose and combinations thereof. The formulation should suit the mode of administration.

Accordingly, the invention provides a use of adipose cell populations, for the manufacture of a medicament to treat kidney disease in a subject. In some embodiments, the medicament may further comprise recombinant polypeptides, such as growth factors, chemokines or cytokines. In further embodiments, the medicaments comprise a human adipose-derived cell population. The cells used to manufacture the medicaments can be isolated, derived, or enriched using any of the variations provided for the methods described herein.

The cell preparation(s), constructs, or compositions are formulated in accordance with routine procedures as a pharmaceutical composition adapted for administration to human beings. Typically, compositions for intravenous administration, intra-arterial administration or administration within the kidney capsule, for example, are solutions in sterile isotonic aqueous buffer. Where necessary, the composition can also include a local anesthetic to ameliorate any pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a cryopreserved concentrate in a hermetically sealed container such as an ampoule indicating the quantity of active agent. When the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there are a wide variety of suitable formulations of pharmaceutical compositions (see, e.g., Alfonso R Gennaro (ed), Remington: The Science and Practice of Pharmacy, formerly Remington's Pharmaceutical Sciences 20th ed., Lippincott, Williams & Wilkins, 2003, incorporated herein by reference in its entirety). The pharmaceutical compositions are generally formulated as sterile, substantially isotonic and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

One aspect of the invention further provides a pharmaceutical formulation, comprising a adipose cell population of the invention, and a pharmaceutically acceptable carrier. In some embodiments, the formulation comprises from 10^4 to 10^9 renal adipose-derived cells.

In one aspect, the present invention provides methods of providing a adipose cell population to a subject in need. In one embodiment, the source of the cell population(s) may be autologous or allogeneic, syngeneic (autogeneic or isogeneic), and any combination thereof. In instances where the source is not autologous, the methods may include the administration of an immunosuppressant agent. Suitable immunosuppressant drugs include, without limitation, azathioprine, cyclophosphamide, mizoribine, ciclosporin, tacrolimus hydrate, chlorambucil, lobenzarit disodium, auranofin, alprostadil, gusperimus hydrochloride, biosynsorb, muromonab, alefacept, pentostatin, daclizumab, sirolimus, mycophenolate mofetil, leflonomide, basiliximab, dornase α, bindarid, cladribine, pimecrolimus, ilodecakin, cedelizumab, efalizumab, everolimus, anisperimus, gavilimomab, faralimomab, clofarabine, rapamycin, siplizumab, saireito, LDP-03, CD4, SR-43551, SK&F-106615, IDEC-114, IDEC-131, FTY-720, TSK-204, LF-080299, A-86281, A-802715, GVH-313, HMR-1279, ZD-7349, IPL-423323, CBP-1011, MT-1345, CNI-1493, CBP-2011, J-695, LJP-920, L-732531, ABX-RB2, AP-1903, IDPS, BMS-205820, BMS-224818, CTLA4-1g, ER-49890, ER-38925, ISAtx-247, RDP-58, PNU-156804, LJP-1082, TMC-95A, TV-4710, PTR-262-MG, and AGI-1096 (see U.S. Patent No. 7,563,822). Those of ordinary skill in the art will appreciate other suitable immunosuppressant drugs.

The treatment methods of the subject invention involve the delivery of an isolated renal adipose-derived cell population into individuals. In one embodiment, direct administration of cells to the site of intended benefit is preferred. In one embodiment, the cell populations of the instant invention are delivered to an individual in a delivery vehicle.

A variety of means for administering cells to subjects will, in view of this specification, be apparent to those of skill in the art. Such methods include injection of the cells into a target site in a subject. Cells can be inserted into a delivery device or vehicle, which facilitates introduction by injection or implantation into the subjects. In certain embodiments, the delivery vehicle can include natural materials. In certain other embodiments, the delivery vehicle can include synthetic materials. In one embodiment, the delivery vehicle provides a structure to mimic or appropriately fit into the organ's architecture. In other embodiments, the delivery vehicle is fluid-like in nature. Such delivery devices can include tubes, e.g., catheters, for injecting cells and fluids into the body of a recipient subject. In a

preferred embodiment, the tubes additionally have a needle, e.g., a syringe, through which the cells of the invention can be introduced into the subject at a desired location. In some embodiments, adipose cell populations are formulated for administration into a blood vessel via a catheter (where the term "catheter" is intended to include any of the various tube-like systems for delivery of substances to a blood vessel). Alternatively, the cells can be inserted into or onto a biomaterial or scaffold, including but not limited to textiles, such as weaves, knits, braids, meshes, and non-wovens, perforated films, sponges and foams, and beads, such as solid or porous beads, microparticles, nanoparticles, and the like (*e.g.*, Cultispher-S gelatin beads - Sigma). The cells can be prepared for delivery in a variety of different forms. For example, the cells can be suspended in a solution or gel. Cells can be mixed with a pharmaceutically acceptable carrier or diluent in which the cells of the invention remain viable. Pharmaceutically acceptable carriers and diluents include saline, aqueous buffer solutions, solvents and/or dispersion media. The use of such carriers and diluents is well known in the art. The solution is preferably sterile and fluid, and will often be isotonic. Preferably, the solution is stable under the conditions of manufacture and storage and preserved against the contaminating action of microorganisms such as bacteria and fungi through the use of, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. One of skill in the art will appreciate that the delivery vehicle used in the delivery of the cell populations thereof of the instant invention can include combinations of the above-mentioned characteristics.

Modes of administration of the isolated renal adipose-derived cell populations, include, but are not limited to, systemic, intra-renal (*e.g.,* parenchymal), intravenous or intra-arterial injection and injection directly into the tissue at the intended site of activity. Additional modes of administration to be used in accordance with the present invention include single or multiple injection(s) via direct laparotomy, via direct laparoscopy, transabdominal, or percutaneous. Still yet additional modes of administration to be used in accordance with the present invention include, for example, retrograde and ureteropelvic infusion. Surgical means of administration include one-step procedures such as, but not limited to, partial nephrectomy and construct implantation, partial nephrectomy, partial pyelectomy, vascularization with omentum ± peritoneum, multifocal biopsy needle tracks, cone or pyramidal, to cylinder, and renal pole-like replacement, as well as two-step procedures including, for example, organoid-internal bioreactor for replanting. In another embodiment, the cell compositions are delivered separately to specific locations or via specific methodologies, either simultaneously or in a temporally-controlled manner, by one or more of the methods described herein.

The appropriate cell implantation dosage in humans can be determined from existing information relating to either the activity of the cells, for example EPO production, or extrapolated from dosing studies conducted in preclinical studies. From *in vitro* culture and *in vivo* animal experiments, the amount of cells can be quantified and used in calculating an appropriate dosage of implanted material. Additionally, the patient can be monitored to determine if additional implantation can be made or implanted material reduced accordingly.

One or more other components can be added to the cell populations, including selected extracellular matrix components, such as one or more types of collagen or hyaluronic acid known in the art, and/or growth factors, platelet-rich plasma and drugs.

Those of ordinary skill in the art will appreciate the various formulations and methods of administration suitable for the cell populations described herein.

8. Kits

The instant invention further includes kits comprising any of the following: the polymeric matrices and scaffolds of the invention and related materials, and/or cell culture media and instructions for use. The instructions for use may contain, for example, instructions for culture of the cells or administration of the cell populations. In one embodiment, the present invention provides a kit comprising a scaffold as described herein and instructions. In yet another embodiment, the kit includes an agent for detection of marker expression, reagents for use of the agent, and instructions for use. This kit may be used for the purpose of determining the expression of one or more biomarkers in a cell population. The kit may also be used to determine the biotherapeutic efficacy of a cell population, admixture, or construct described herein.

9. Reports

The methods of this invention, when practiced for commercial purposes generally produce a report or summary related to characteristics of a adipose cell population. The report may include information on any defining feature of the cell population described herein. The methods and reports of this invention can further include storing the report in a database. Alternatively, the method can further create a record in a database for the subject and populate the record with data. In one embodiment the report is a paper report, in another embodiment the report is an auditory report, in another embodiment the report is an electronic record. It is contemplated that the report is provided to a physician and/or the patient. The receiving of the report can further include establishing a network connection to a server computer that includes the data and report and requesting the data and report from the server computer. The methods provided by the present invention may also be automated in whole or in part.

All patent, patent application, and literature references cited in the present specification are hereby incorporated by reference in their entirety.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

EXAMPLES

EXAMPLE 1 - Regulated Expression of Erythropoietic, Angiogenic, and Developmental Factors in Renal Adipose

We investigated whether cells isolated from the stromal vascular fraction (SVF) of adipose could potentially influence erythropoiesis and angiogenesis *in vivo* by studying the *in vitro* expression of

erythropoietin (EPO) and vascular endothelial growth factor (VEGF) in cells derived from renal and non-renal SVF sources and expanded in α-MEM culture media with 10% fetal bovine serum (SVF cells). Expression of both EPO and VEGF mRNA isolated from SVF cells was regulated by hypoxia. Although oxygen-regulated expression of EPO has been documented for liver, kidney and brain derived cells, the expression of EPO by adipose SVF cells reported here was comparable to that of primary kidney cells or hepatocytes. Iso-electric focusing revealed that patterns of post-translational processing of EPO mRNA were different in SVF cells derived from renal and non-renal adipose, suggesting that renal and non-renal adipose tissue are functionally distinct. SVF cells from renal adipose also specifically expressed the transcription factor Wilms tumor 1 (WT1) that are involved in renal organ development during embryogenesis. Taken together, these data are consistent with the notion that kidney sourced adipose stromal (KiSAS) cells may be primed to recreate a regenerative micro-environment within the kidney. These findings open the possibility of isolating solid-organ associated adipose derived cell populations for therapeutic applications in organ-specific regenerative medicine products and could potentially represent a novel source for therapeutic factors for treating anemia secondary to decreased EPO expression.

Here, we have focused in the current study on evaluation of key functional criteria discriminating stromal cells derived from kidney and non-kidney sourced adiposes through analysis of established regenerative and developmental markers associated with kidney: erythropoietin (EPO), VEGF and WT1. We demonstrate for the first time that renal adipose tissue presents depot specific expression of EPO, and that stromal cell populations derived from kidney and non-kidney sourced adiposes express EPO and VEGF in a hypoxia-regulated manner. Furthermore, we show that expression of the key nephrogenic transcription factor WT1 is specific to kidney adipose sourced stromal cells, and that niche specific adipose depots within kidney may be defined by distinctive WT1 transcriptional splice variants. Taken together, these data extend the concept of functionally unique, location-specific adipose depots from the systemic to the organ-level, and establish a foundation for application of kidney sourced adipose as an alternate cell source for tissue engineering and regenerative therapies of the kidney.

MATERIALS and METHODS

Isolation of renal and non-renal adipose-derived cells. Human non-renal adipose was obtained either subcutaneously or through visceral liposuction (Zen-Bio, www.zen-bio.com). Renal adipose from the renal pedicle was dissected away from human kidney (normal adult) donated through National Disease Research Institute (NDRI) in compliance with all NIH guidelines governing the use of human tissues for research purposes. Renal adipose from the major calyx was obtained by bisection of whole human kidney and dissection of calyx fat away from the medulla. Canine kidneys for isolation of canine renal adipose were obtained from the University of North Carolina at Chapel Hill (gift of Dr. Timothy Nichols). Rat kidneys for isolation of rat visceral and organ associated adiposes were sourced from male Lewis rats obtained from Charles River Labs. Regardless of species or tissue origin, all adipose samples were processed as follows: Adipose was extensively washed with PBS/0.1% gentamicin

(Invitrogen-Gibco) and digested for up to 1 hour with 0.3% collagenase I (Worthington), 1% BSA in DMEM-HG (Invitrogen-Gibco) at 37° C. Samples were centrifuged at 600*g* for 20 minutes and the adipocytic supernatant aspirated away. The remaining stromal vascular fraction was re-suspended in α-MEM/10% FBS (Invitrogen-Gibco) and placed in a tissue culture incubator for 24-48 hours. Non-adherent cell populations were removed by washing 3X with PBS. For experiments involving hypoxic inductions, cells were maintained in an O_2-enriched (2%) incubator for the time periods indicated. VEGF mRNA expression was used as a control to confirm integrity of hypoxic regulation pathways.

Non-Adipose Cells. Renal primary kidney cells from rat or human kidneys were isolated as previously described (Basu et al. Cell Transplant 2011 Mar 24. [Epub ahead of print]; Presnell SC et al. Tissue Eng Part C Methods, 17 (3): 261-273, 2011). Human peripheral blood derived mononuclear cells were isolated as described (Spector, D.L. Cells, a laboratory manual. Cold Spring Harbor Press, 1997). CD34+ GCSF mobilized/non-mobilized peripheral blood mononuclear cell cDNA was purchased from AllCells LLC. Fetal and adult hepatocyte cDNA and keratinocyte cDNA were purchased from ScienCell Research Laboratories.

TaqMan qRT-PCR. RNA was purified from cell samples using the RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's instructions. cDNA was generated from the entire final volume of RNA using the SuperScript VILO cDNA Synthesis Kit (Invitrogen-Gibco) according to the manufacturers' instructions. Following cDNA synthesis, each sample was diluted 1:10 and used directly to set up qRT-PCR as follows: 10 µl master mix (2X), 1 µl primer/probe, 9 µl cDNA. The following TaqMan primer/probe sets were procured from Applied Biosystems: rat EPO: Rn01481376_m1, Rat VEGF-A: Rn00582935, human EPO: Hs00171267_m1, human VEGF-A: Hs00900058_m1, human WT-1: Hs01103754_m1. Primer/probe sets were also procured for cubulin, NPHSI, E-cadherin, podocalyxin, VitD hydroxylase and Wnt4. All TaqMan reactions were carried out in an ABI 7300 real time thermal cycler using default cycling parameters. Analysis of PCR data was performed using the method of Relative Quantitation (RQ) by comparative Ct.

Iso-electric focusing gel analysis of EPO. Up to 1 X 10^6 cells or up to 30mg total adipose tissue was lysed in protein lysis buffer (50mM Tris pH 8.0; 150mM NaCl; 0.5% NP40 and protease inhibitors, Roche). 10µg of protein lysate was loaded onto a pH 3-7 iso-electric focusing (IEF) gel (Invitrogen-Gibco) and run out as recommended by the manufacturer. IEF gels were transferred to nitrocellulose using the iBlot transfer system (Invitrogen-Gibco) and probed overnight with MAB 2871 anti-hEPO monoclonal antibody (R&D Systems) at 1/500 in TBST (Tris-buffered saline, pH 7.0, 0.1% Tween-20)/2% milk. Secondary antibody was horse anti-mouse IgG/HRP conjugate (Vector Labs) at 1/60000 in TBST/2% milk.

FACs and immuno-fluorescence. Cells (0.5 x 10^6- 1 x 10^6) were fixed in 2% para-formaldehyde and F_c receptors blocked to prevent non-specific binding. Cells were permeabilized by incubation in PBS/0.2% Triton X-100/10% horse serum for 30 minutes. Cells were then incubated with a directly conjugated antibody against human WT1 (Santa Cruz) as recommended by the manufacturer. Subsequent to final washing (PBS, 0.2% Triton X-100), antigen detection was performed utilizing the Guava EasyCyte Mini Express Assay system using the appropriate fluorescent channel. A minimum of 5,000-10,000 events were acquired from each sample. For immuno-fluorescence analysis, the labeled cell suspension was centrifuged directly onto a poly-L-lysine coated slide (Electron Microscopy Sciences) using a cyto-centrifugation system (Viescor) at 1,500 rpm for 5 minutes. Cells were counterstained with DAPI containing mounting medium (Vector Labs) and viewed with a Leica DMI 4000B fluorescence microscope.

RESULTS

EPO is expressed by multiple cell sources. To evaluate expression of EPO mRNA from adipose-derived stromal cells relative to established sources of EPO, TaqMan quantitative RT-PCR was performed. Human primary renal cells were used as calibrator and have a designated RQ value of 1.0. Samples are as follows: H_2O as a negative control (1); human primary renal cells (2); whole fetal liver (3); CD34+ enriched fetal liver cells (4); hepatocytes (5); keratinocytes at passage 4 (6); human dermal microvascular endothelial cells (7); human epidermal keratinocytes (8); peripheral blood derived mononuclear cells, CD34+, GCSF mobilized (9); peripheral blood derived mononuclear cells, CD34+, normal (10); adipose (lipoaspirate) stromal cells at passage 0 (11); passage 1 (12); passage 2 (13); passage 4 (14); adipose (subcutaneous) stromal cells at passage 0 (15); passage 1 (16); passage 2 (17); passage 3 (18); and passage 4 (19).As shown in **Figure 1A**, strong expression of EPO is observable from renal primary cells (cell type 2), fetal liver (cell type 3), adult hepatocytes (cell type 5), keratinocytes (cell types 8 and 8) and non-mobilized CD34+ PBMNCs (cell type 10). Mobilization of CD34+ PBMNCs with GCSF was observed to lead to silencing of EPO mRNA expression (cell type 9). Relatively lower levels of EPO mRNA were observed from multiple samples of non-kidney sourced adipose stromal cells such as CD34+ enriched fetal liver cells (cell type 4) as well as passaged lipoaspirate (cell types 11-14) and subcutaneous (cell types 15-10) adipose stromal cells. EPO expression was also evaluated from kidney and non-kidney sourced adipose stromal cells directly against renal primary cells. Samples are as follows: Primary renal cells (1 and 2); primary renal cells exposed to hypoxic conditions (3); adipose lipoaspirate (4) and subcutaneous (5) stromal cells at passage 0; adipose lipoaspirate (6) and subcutaneous (7) stromal cells at passage 1; major calyx derived kidney sourced adipose stromal cells at passage 1 (8); renal pedicle derived kidney sourced adipose stromal cells at passage 1 (9) and passage 3 (10); fetal hepatocytes used as a positive control (11); H_2O used as a negative control (12).As shown in **Figure 1B**, expression of EPO mRNA from both primary kidney cells (cell types 1- 3) and non-kidney sourced adipose stromal cells (cell types 3-7, 9, 10) is comparable to that observed from primary kidney cells.

Morphological features of renal adipose-derived cells are dependent on the site of isolation at the kidney. We isolated adipose derived from the renal pedicle as well as from the major calyx compartments of the kidney. Adipose cells derived from the major calyx have a distinctly endothelial morphology compared to adipose cells obtained from the renal pedicle or from non-renal sources, which have a noticeably more fibroblastic appearance.

Expression of EPO mRNA from kidney and non-kidney sourced adipose stromal cells is regulated by hypoxia. A key characteristic of EPO expression from kidney is regulation by environmental oxygen (Sytkowski, A.J. Erythropoietin. Blood, brain and beyond. Wiley, 2004, Gleadle, J.M. Nephrology **14**, 86, 2006). Based on this criterion, an experiment was performed to establish whether expression of EPO from adipose stromal cells was physiologically relevant by quantifying the production of EPO mRNA under conditions of high and low environmental oxygen. Initial studies focused on rodents, as the rat is the small animal model of choice for the investigation of CKD (Ueda et al. Life Sci **84**, 853, 2009). Both EPO and VEGF expression are up-regulated in response to hypoxia within 4 hours, and returns towards baseline within 48 hours of transfer to normoxia (n=3). As shown in **Figure 2A**, rat visceral adipose stromal cells respond to hypoxia by an up-regulation of the expression of EPO mRNA within 4 hours of treatment. Expression of VEGF mRNA from rat visceral adipose stromal cells is also tightly controlled by hypoxia, showing up-regulation within 4 hours of treatment and subsequent down-regulation within 48 hours of return to normoxia (**Figure 2B**). This result is reflected by human KiSAS cells. EPO expression is up-regulated in cells from both isolates in response to hypoxia within 8 hours, and returns towards baseline within 48 hours of transfer to normoxia (n=3). As shown in **Figure 3A-D**, hypoxia induced up-regulation of EPO and VEGF mRNA expression was observed from human renal pedicle and major calyx sourced adipose stromal cells. VEGF expression is up-regulated in cells from both isolates in response to hypoxia within 4 hours, and returns towards baseline within 48 hours of transfer to normoxia (n=3).

Expression of EPO protein is comparable between human KiSAS cells and human renal primary cells. In addition to our finding that expression levels of EPO mRNA in adipose stromal cells are significantly lower relative to primary renal cells, we also wanted to quantitate EPO from a functional or bio-therapeutic perspective by evaluating EPO protein expression between differently sourced adiposes. We therefore investigated expression of EPO protein from adipose sourced stromal cells using iso-electric focusing gels (IEF). IEF technology has been used to discriminate between multiple, differently modified isoforms of EPO with high resolution (Lasne, F. and de Ceaurriz J. Nature **405**, 635, 2000). Kidney and non-kidney sourced adipose stromal cells express distinct isoforms of EPO distinguishable though isoelectric focusing gel electrophoresis and western blotting. Samples are as follows: human keratinocytes (1), hepatocytes (2), renal- (3) non-renal- (4) adipose stromal- cells and primary renal-cells all under normoxia (5), primary renal cells under hypoxia (6) and HepG2 cells as a

positive control (7). Blot was probed with anti-EPO monoclonal antibody for Western analysis. Comparison of lanes 5 and 6 shows clear up-regulation of EPO protein expression in response to hypoxia from primary renal cell isolates. All lanes were normalized by total mass of protein (10μg). **Figure 4A** demonstrates that EPO protein is expressed from human KiSAS cells (lane 3) at levels directly comparable to that observed from established cell sources of EPO, including keratinocytes (lane 1), hepatocytes (lane 2), and primary kidney cells (lanes 5, 6) (Bodo et al. FASEB J **21**, 3346, 2007, Weidemann, A. and Johnson, R.S. Kidney Int **75**, 682, 2009). EPO is also expressed by visceral (non-renal) adipose stromal cells (lane 4), but at significantly reduced levels. Kidney and non-kidney sourced adipose stromal cells show distinctive patterns of post-translational EPO modification resulting in unique migration profiles on IEF gels, as can be seen by comparing lane 3 with lane 4. In addition, EPO from kidney or non-kidney sourced adipose stromal cell sources is further distinguishable from EPO expressed by primary renal cells on the basis of iso-electric point (compare lanes 3 and 4 with lanes 5 and 6). Finally, EPO isoforms expressed by human keratinocytes and hepatocytes (lanes 1 and 2) are distinguishable from all other cell sources.

As dog is the large animal model of choice for evaluation of renal cell therapies (Lee et al. Blood Purif **26**, 491, 2008), we examined expression of EPO protein in canine kidney sourced adipose stromal cells and primary renal cells using IEF gels. Samples are as follows: canine primary renal cells under normoxia (1) and hypoxia (2), major calyx-derived adipose stromal cells (3), renal pedicle derived-adipose stromal cells (4) and recombinant canine EPO as a positive control (5). Blot was probed with anti-EPO monoclonal antibody for Western analysis. All lanes were normalized by total mass of protein (10μg). **Figure 4B** demonstrates that canine KiSAS cells sourced from either major calyx adipose (lane 3) or renal pedicle adipose (lane 4) express EPO protein at levels comparable to that observed from canine primary renal cells (lanes 1 and 2). Additionally, as is the case for human adipose, EPO expressed from stromal cells sourced from either the renal pedicle or major calyx have unique IEF signatures that discriminate canine adipose sourced EPO from that expressed by canine primary renal cells. Taken together, these data establish that KiSAS cells express EPO protein at levels comparable to that seen from established cellular sources of EPO including renal cells, hepatocytes and keratinocytes and confirm that differently sourced EPO may be identified by iso-electric point profiling (Lasne, F. and de Ceaurriz J. Nature **405**, 635, 2000; Bodo et al. FASEB J **21**, 3346, 2007; Weidemann, A. and Johnson, R.S. Kidney Int **75**, 682, 2009).

Kidney sourced adipose tissue is an organ-specific reservoir for EPO. To establish if expression of EPO from adipose stromal cell populations reflects a physiologically relevant EPO reservoir *in vivo*, we examined expression of EPO protein from differently sourced rat whole adipose tissue by IEF. Adipose tissue is a reservoir for EPO expressing cells distinguishable though isoelectric focusing gel electrophoresis and western blotting. White and brown adipose are derived from visceral depot. All lanes normalized by mass of protein to 10μg. Table shows quantitative densitometric analysis of EPO expression expressed as band intensity per unit gel area. As shown in **Figure 5** where individual

lanes are normalized by mass protein loaded, robust expression of EPO is specifically associated with kidney sourced adipose tissue. Although EPO is detectable from non-kidney organ sources of adipose tissue such as liver and heart, expression is 5-fold higher in kidney sourced adipose over liver sourced adipose and 2.8-fold higher in kidney sourced adipose over heart sourced adipose. Interestingly, comparison of white and brown adipose sourced from visceral adipose depots shows EPO expression is 5-fold higher in white adipose over brown adipose.

Expression of the developmental transcription factor WT1 distinguishes kidney from non-kidney sourced adipose stromal cells. We reasoned that renal markers other than EPO might be associated with organ specific expression patterns within differently sourced adiposes. WT1 is a key zinc finger transcription factor broadly involved in organogenesis. WT1 acts to modulate the earliest stages of nephrogenesis, and may serve as a marker for regeneration (Roberts, S.G. Curr Opin Genet Dev **15**, 542, 2005; Litbarg et al. Cell Tissue Res. **328**, 487, 2007; Zhou et al. Nature **454**, 109, 2008). We used primers specific to the KTS+ and KTS- transcriptional splice variants of WT1 (Hammes et al. Cell **106**, 319, 2001) to investigate the expression of WT1 within kidney and non-kidney sourced adipose stromal cells. As shown in **Figure 6A**, expression of WT1 mRNA is specific to kidney sourced adipose stromal cells (lanes 4 and 5), with no expression being observed from visceral adipose stromal cells (lanes 2 and 3). Interestingly, the ratio of these two splice variants differs between stromal cells sourced from major calyx adipose (lane 4) or renal pedicle adipose (lane 5) derived from the same donor. All lanes were normalized by total mass of cDNA: molecular weight ladder used for sizing (1), lipoaspirate stromal cells (Lane 2), subcutaneous adipose stromal cells (Lane 3), major calyx adipose stromal cells (Lane 4), renal pedicle stromal cells (Lane 5). Expression of WT1 is only detectable from renal adipose. Note that the ratio of KTS+/KTS- splice variants differs between major calyx and renal pedicle derived cell sources derived from the same donor.

We extended the WT1 RT-PCR analysis by evaluating expression of WT1 protein in KiSAS cells through FACs and immuno-fluorescence approaches. **Figure 6B and C** shows that both renal pedicle and major calyx sourced adipose stromal cells have a WT1+ population, ranging from approximately 45% (renal pedicle) to 52% (major calyx) of the population. 45.6% of renal pedicle adipose stromal cell population was WT1+. 52.4% of major calyx adipose stromal cell population was WT1+. Expression of WT1 is both nuclear and cytoplasmic, (**Figure 6D**), as has been previously reported (Niksic et al. Hum Mol Gen **13**, 463, 2004). Note that localization of WT1 expression is cytoplasmic and nuclear. WT1 (green), DNA (red).

Renal adipose-derived cells specifically express multiple markers associated with nephrogenesis. We further extended the gene expression analysis by performing TaqMan QRT-PCR to quantitatively evaluate the expression of additional renal markers associated with nephrogenesis from renal and nonrenal adipose-derived cells. Major calyx adipose-derived stromal cells and renal pedicle-derived stromal cells derived from the same donor were analyzed. We found that the renal adipose-

derived cells express the key nephrogenic transcription factor WT1. WT1 modulates expression of GDNF, a secreted signaling factor capable of triggering ureteric bud formation (Brodbeck & Englert, Pediatr Nephrol. 2004 Mar;19(3):249-55). The expression of WTI from renal adipose-derived cells was found to be significantly below that observed for renal primary cells. However, expression was not observed at any level from non-renal adipose-derived cells.

By QRT-PCR, we also found that the renal adipose-derived cells express kidney tubular cell biomarkers including cubulin, NPHSI, E-cadherin, podocalyxin, VitD hydroxylase and Wnt4. NPHSI is a cell adhesion protein resident in glomerular podocytes (Ruotsalainen et al., Am J Pathol 157(2000): 1905-16). E-cadherin is a marker of tubular epithelial cells that functions in the maintenance of apicobasal polarity (Halbleib & Nelson, Genes Dev. 2006 Dec 1;20(23):3199-214). Non-renal adipose cells were analyzed for expression of E-cadherin and NPHSI and found not to express these tubular cell biomarkers.

These studies established a baseline of renal marker expression for preliminary attempts to induce the acquisition of a tubular phenotype through directed differentiation strategies. To this end, methodologies for the directed differentiation of progenitor populations towards a renal phenotype have been described for both embryonic stem cells and adult-derived stem cells. For example, application of activin-A and retinoic acid was sufficient to induce expression of multiple early renal developmental markers including WT1, Wnt4 and GDNF in murine embryoid bodies (Kim & Dressler, J Am Soc Nephrol 16(2005): 3527-34). We observed up-regulation of podocalyxin expression from renal adipose-derived cells in response to 10μM retinoic acid. Additionally, levels of the glomerular marker podocalyxin were elevated in non-renal adipose-derived cells in response to treatment with bone morphogenic proteins (BMPs).

In the current report, we show that kidney sourced adipose represents a hitherto unidentified reservoir of EPO producing cells. Observed down-regulation of EPO mRNA expression during the hypoxia-normoxia transition is not as tightly regulated as that shown by VEGF mRNA (**Figures 1-3**), despite both VEGF and EPO mRNAs being responsive to hypoxia through the same HIF-1α/2α mediated regulatory pathways (Gleadle, J.M. Nephrology **14**, 86, 2006). This apparently less stringent regulatory control of EPO mRNA is likely due to the significantly lower relative expression levels of EPO mRNA compared to VEGF mRNA (Plotkin, M.D. and Goligorsky, M.S. Am J Physiol Renal Physiol **291**, 902, 2006). That expression of EPO is not an artifact generated from cell culture is demonstrated by the observation that kidney sourced adipose tissue is a reservoir for EPO expression (**Figure 5**). Interestingly, **Figure 5** also shows that although EPO expression from non-kidney sourced adipose is significantly lower relative to kidney sourced adipose, EPO is nevertheless a specific marker within visceral sourced adipose discriminating white from brown adipocytes. As shown in **Figure 4A**, expression of EPO from KiSAS cells is equivalent to that observed from primary renal cell populations currently under development for cell therapy of anemias secondary to CKD (Aboushwareb, T. et al. World J Urol **26**, 295, 2008), suggesting that kidney sourced adipose may represent an alternate cell source for cellular vectors for EPO delivery.

Variation in the iso-electric isotype signature of EPO within urine and corresponding serum has been previously documented (Lasne, F. et al. Int J Biol Macromol **41**, 354, 2007). Iso-electric isotype profiling has been used to discriminate recombinant from native EPO in urine of athletes suspected of illicit self-medication (Weidemann, A. and Johnson, R.S. Kidney Int **75**, 682, 2009). Similarly, IEF gel electrophoresis analysis of stromal cell populations sourced from kidney and non-kidney depots shows that such cell types may be functionally distinguished by unique differences in the pattern of post-translational modification of EPO (**Figure 4A**). Furthermore, additional iso-electric point isotypes of EPO are observable within differently sourced adiposes originating from the same organ, as demonstrated by the distinctive IEF signatures of EPO expressed by major calyx or renal pedicle-sourced adipose stromal cells (**Figure 4B**). This data lends credence to the notion that major calyx and renal pedicle represent distinct adipose depots within kidney.

Our observations on the differential post-translational modification patterns of EPO expressed by kidney and non-kidney sourced adipose stromal cells led us to investigate the expression status of additional key renal markers that could potentially serve to further distinguish between renal and non-renal adipose depots. To this end, WT1 is a zinc finger transcription factor broadly involved in organogenesis. WT1 acts to modulate the earliest stages of nephrogenesis, and may serve as a marker for regeneration (Roberts, S.G. Curr Opin Genet Dev **15**, 542, 2005; Litbarg et al. Cell Tissue Res. **328**, 487, 2007; Zhou et al. Nature **454**, 109, 2008). Expression of WT1 mRNA is specific to kidney sourced adipose stromal cells (**Figure 6A**). WT1 mRNA is not detected from viscerally sourced adipose. Transcriptional regulation of WT1 is complex and multiple splice variants with distinctive biological functions have been characterized. The KTS+ and KTS- variants of WT1 differ in the presence or absence of 3 amino acids (KTS) between zinc fingers 3 and 4. The ratio of KTS+/KTS- WT1 splice variants is reported to be constant across multiple tissues and the manipulation of this balance can trigger severe developmental anomalies (Hammes, A. et al. Cell **106**, 319, 2001). The observation that kidney sourced adipose stromal cells (but *not* non-kidney sourced adipose stromal cells) specifically express WT1 is consistent with observed differences in expression levels and in post-translational modification of EPO between kidney and non-kidney sourced adipose stromal cells (**Figure 4B**). Observed differences in the ratio of KTS+/KTS- WT1 splice variants within differently sourced adipose stromal cells derived from the same organ of the same individual is remarkable; such disparity is contrary to previous reports (Hammes, A. et al. Cell **106**, 319, 2001), but is consistent with organ and niche specific variability in EPO IEF signatures documented herein, providing further confirmation of depot specific functionalities within adipose. Interestingly, only approximately 50% of the stromal cell population derived from either major calyx or renal pedicle depots is WT1+ (**Figure 6B and C**), suggesting that two functionally distinct cellular sub-populations may be present within these adipose tissues.

In conclusion, we show in this report that adipose tissue is a novel source of EPO. We demonstrate that expression of EPO and VEGF mRNAs from adipose (kidney and non-kidney sources) is regulated by environmental oxygen and is directly comparable to that observed from primary renal cells or other established sources of EPO. Renal and non-renal sources of adipose have unique functional

properties manifested through differences in the level of expression and post-translational modification of EPO. These data suggest that renal and non-renal adipose represent fundamentally distinct adipose depots. To this end, EPO protein expressed by kidney and non-kidney sourced adipose stromal cells is distinguishable based on distinctive migration patterns through IEF gels, a consequence of differences in the post-translational modification of EPO between the two cell types. Furthermore, KiSAS cells recapitulate several additional aspects of the functional phenotype of primary renal cells including expression of the key nephrogenic transcription factor WT1. It is possible that renal adipose may be amenable towards acquisition of tubular functionality. In this regard, we have observed induction of established tubular markers within primary cultures of KiSAS cells in response to modulation with known morphogenic agents such as retinoic acid (our unpublished observations). Renal adipose may represent a potentially ideal alternate cell source for treatment of chronic anemia secondary to CKD, since renal adipose is isolatable in much larger quantities than renal primary cells and, as is the case for adipose associated with tubular organs such as bladder, may be unaffected by the occurrence of metastasis in renal cancer patients (Jenkins, M.A. and Munch, L.C. Urology **59**, 444, 2002; Genheimer, C.W. et al. Appl Immunohistochem Mol Morphol **19**, 184, 2011). Taken together, the properties of the renal adipose-derived cells (e.g., regulatable EPO and VEGF production, expression of a key nephrogenic transcription factor WT1, indicate that a renal adipose-derived cell population may contribute towards therapeutic engraftment at the site of injury or facilitate the creation of a regenerative micro-environment within the diseased kidney.

WHAT IS CLAIMED IS:

1. An isolated adipose cell population which expresses erythropoietin (EPO).

2. The cell population of claim 1, derived from renal adipose tissue.

3. The cell population of claim 1, derived from an adipose tissue selected from the group consisting of heart adipose, liver adipose, subcutaneous adipose, visceral adipose, white adipose, brown adipose.

4. The cell population of claim 2, derived from renal pedicle adipose tissue.

5. The cell population of claim 2, derived from renal calyx adipose tissue.

6. The cell population of any one of claims 1-5, derived from an adipose stromal vascular fraction (SVF).

7. The cell population of any one of claims 1-5, which further expresses vascular endothelial growth factor (VEGF).

8. The cell population of any one of claims 1-5, wherein EPO expression is hypoxia-regulated expression.

9. The cell population of any one of claims 1-5, wherein VEGF expression is hypoxia-regulated expression.

10. The cell population of any one of claims 1-5, wherein EPO transcript is expressed.

11. The cell population of any one of claims 1-5, wherein VEGF transcript is expressed.

12. The cell population of any one of claims 1-5, wherein EPO polypeptide is expressed.

13. The cell population of any one of claims 1-5, wherein EPO transcript and EPO polypeptide are expressed.

14. The cell population of claim 1 or 2, which expresses an EPO polypeptide comprising a post-translational modification different from a non-adipose cell population.

15. The cell population of claim 14, wherein the non-adipose cell population is selected from the group consisting of keratinocytes, hepatocytes, and primary kidney cells.

16. The cell population of claim 2, which expresses an EPO polypeptide comprising a post-translational modification different from a non-renal adipose cell population.

17. The cell population of claim 16, wherein the non-renal adipose cell population is selected from the group consisting of keratinocytes, hepatocytes, visceral adipose stromal cells, and primary kidney cells.

18. The cell population of any one of claims 2, 4, and 5, which differentially expresses a biomarker associated with renal regeneration.

19. The cell population of claim 18, wherein the differential expression is increased expression.

20. The cell population of claim 18 or 19, wherein the biomarker is WT-1.

21. The cell population of claim 20, wherein WT-1 transcript is expressed.

22. The cell population of claim 21, wherein the WT-1 transcript is WT-1 KTS^+.

23. The cell population of claim 21, wherein the WT-1 transcript is WT-1 KTS^-.

24. The cell population of claim 21, wherein the WT-1 transcript is WT-1 KTS^+ and WT-1 KTS^-.

25. The cell population of claim 18, wherein the biomarker is a WT-1 polypeptide.

26. A method of preparing an erythropoietin (EPO)-expressing adipose stromal cell population, comprising

a) digesting adipose tissue ; and

b) depleting the digested tissue of adipocytes to provide a stromal vascular fraction (SVF), wherein the SVF comprises the EPO-expressing adipose stromal cell population.

27. The method of claim 26, wherein the EPO-expressing adipose stromal cell population is the cell population of any one of claims 1 to 25.

28. An implantable construct for providing improved kidney function to a subject in need comprising:

a) a biocompatible matrix; and

b) an adipose cell population which express erythropoietin (EPO) deposited on or embedded in a surface of the matrix.

29. The implantable construct of claim 28, wherein the adipose cell population is the cell population of any one of claims 1 to 25.

30. A method of treating a kidney disease in a subject in need, the method comprising administering to the subject a composition comprising an adipose cell population which express erythropoietin (EPO).

31. The method of claim 30, wherein the adipose cell population is the cell population of any one of claims 1 to 25.

32. A method of treating a kidney disease in a subject in need, the method comprising administering to the subject an implantable construct comprising:

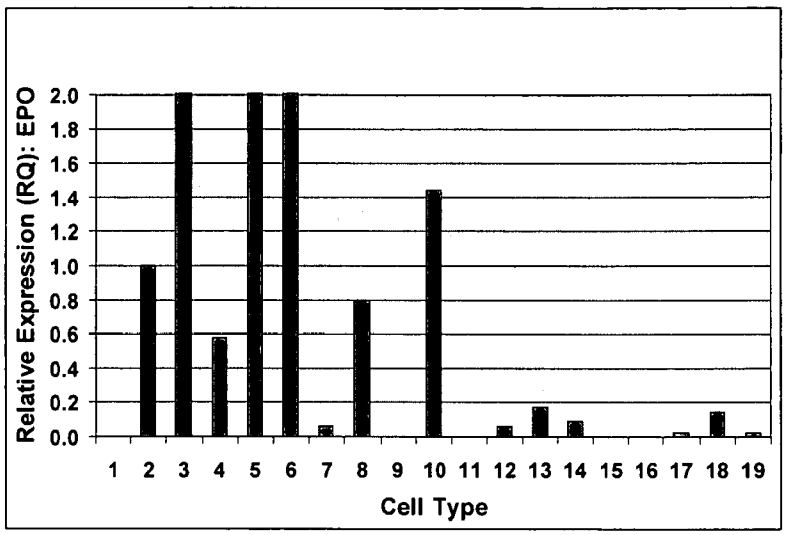
a) a biocompatible matrix; and

b) an adipose cell population which express erythropoietin (EPO) deposited on or embedded in a surface of the matrix.

33. The method of claim 32, wherein the adipose cell population is the cell population of any one of claims 1 to 25.

Figure 1.

A.


Relative Expression (RQ): EPO
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Cell Type

B.

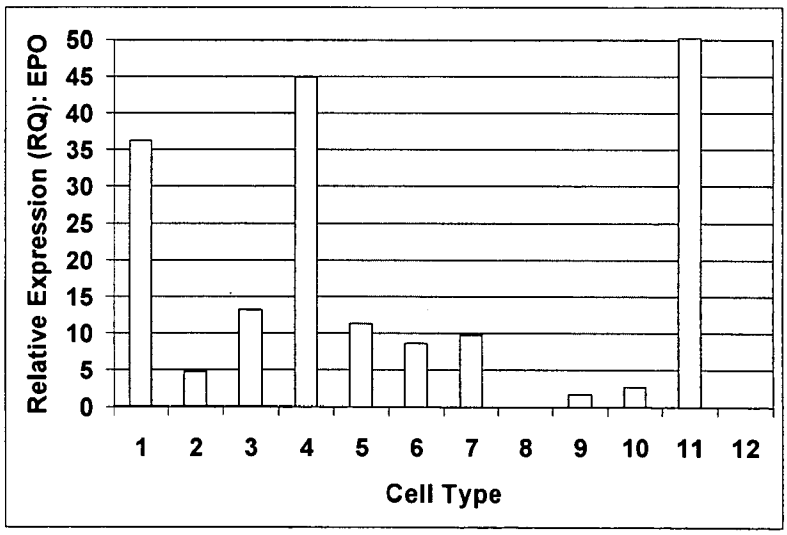
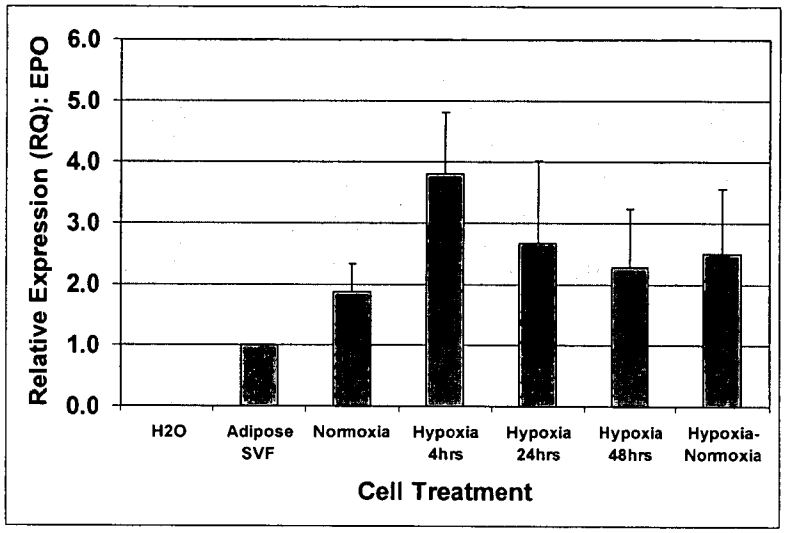

Relative Expression (RQ): EPO
50
45
40
35
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12
Cell Type

Figure 2.

A.


Relative Expression (RQ): EPO
6.0
5.0
4.0
3.0
2.0
1.0
0.0
H2O
Adipose SVF
Normoxia
Hypoxia 4hrs
Hypoxia 24hrs
Hypoxia 48hrs
Hypoxia-Normoxia
Cell Treatment

B.

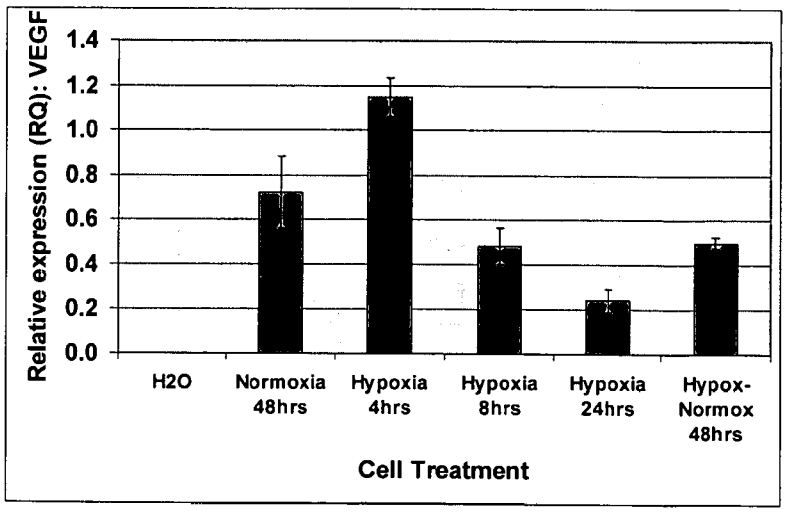
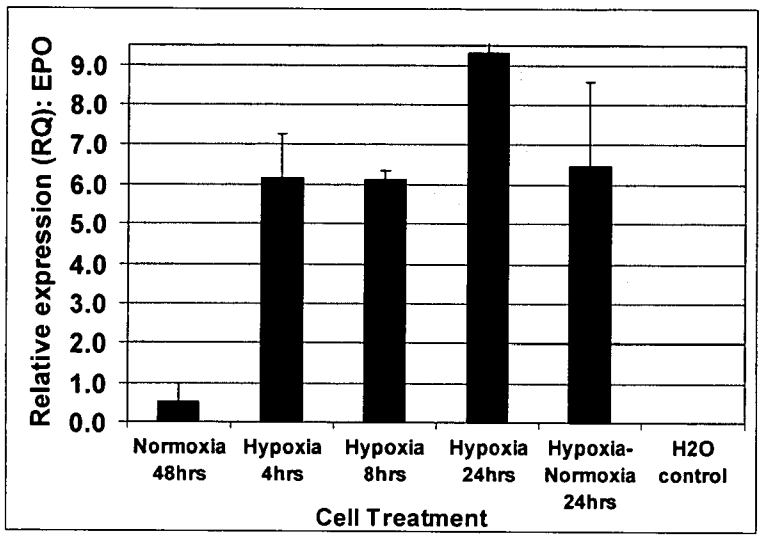
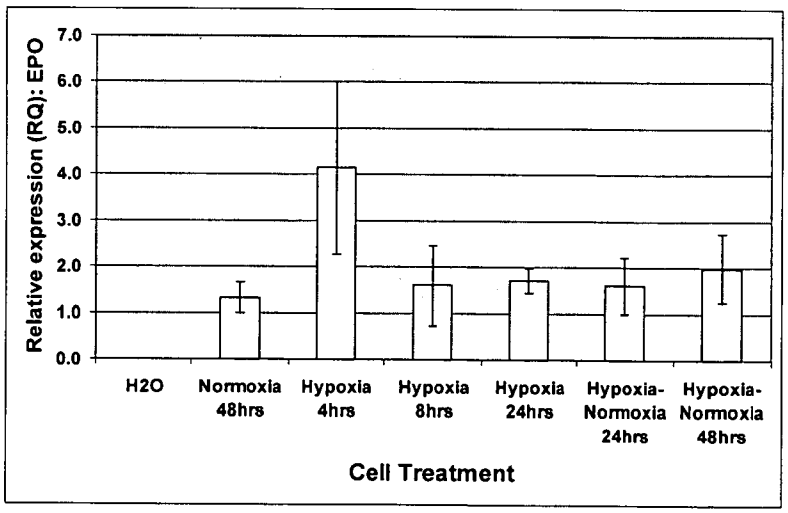

Relative expression (RQ): VEGF
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
H2O
Normoxia 48hrs
Hypoxia 4hrs
Hypoxia 8hrs
Hypoxia 24hrs
Hypox-Normox 48hrs
Cell Treatment

Figure 3.

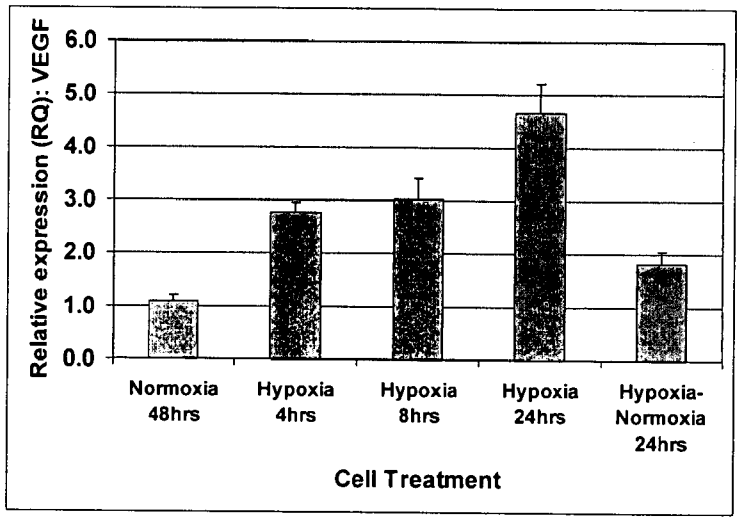
A.


Relative expression (RQ): EPO
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Normoxia 48hrs
Hypoxia 4hrs
Hypoxia 8hrs
Hypoxia 24hrs
Hypoxia-Normoxia 24hrs
H2O control
Cell Treatment

B.


Relative expression (RQ): EPO
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
H2O
Normoxia 48hrs
Hypoxia 4hrs
Hypoxia 8hrs
Hypoxia 24hrs
Hypoxia-Normoxia 24hrs
Hypoxia-Normoxia 48hrs
Cell Treatment

C.


Relative expression (RQ): VEGF
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Normoxia 48hrs
Hypoxia 4hrs
Hypoxia 8hrs
Hypoxia 24hrs
Hypoxia-Normoxia 24hrs
Cell Treatment

D.

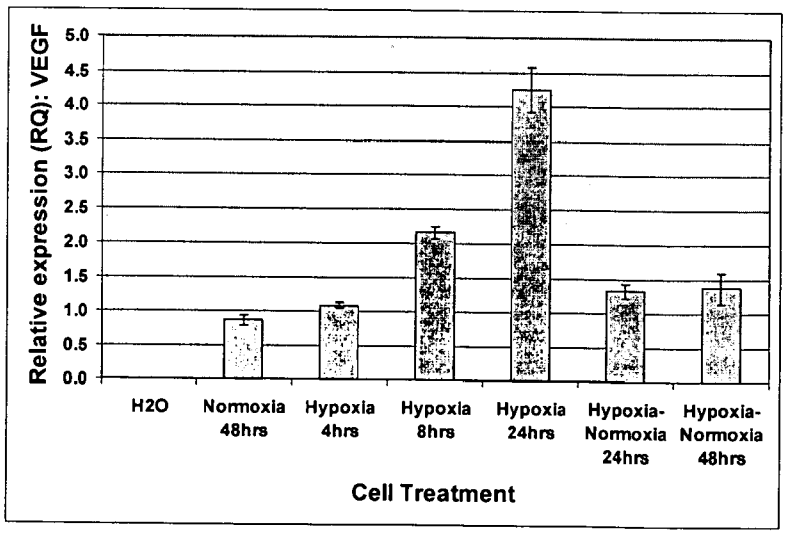
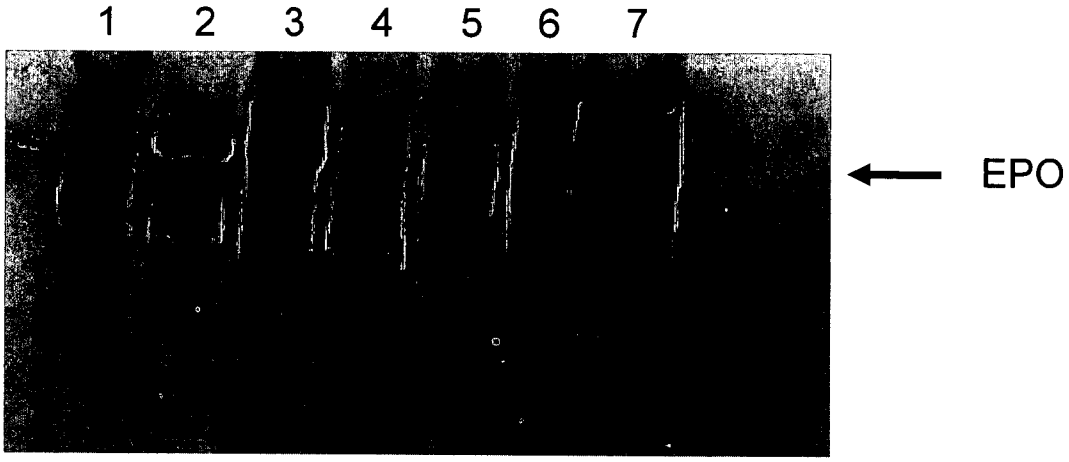

Relative expression (RQ): VEGF
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
H2O
Normoxia 48hrs
Hypoxia 4hrs
Hypoxia 8hrs
Hypoxia 24hrs
Hypoxia-Normoxia 24hrs
Hypoxia-Normoxia 48hrs
Cell Treatment

Figure 4.

A.



B.

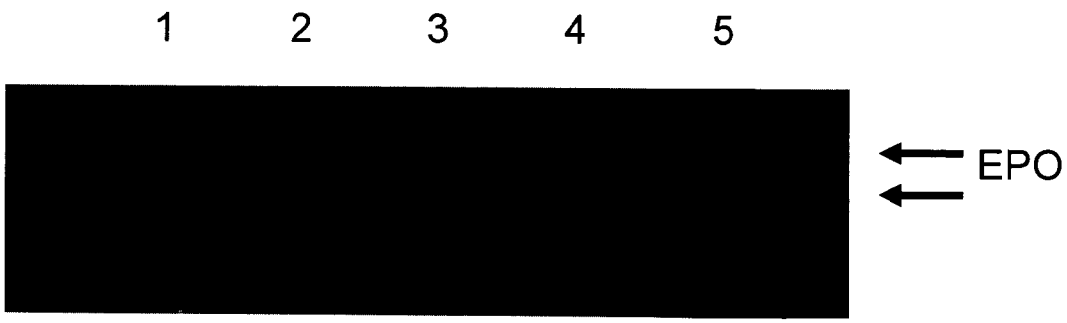
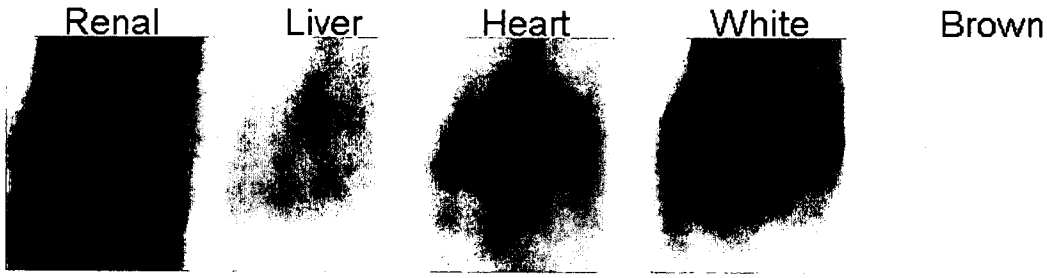


Figure 5.



Source	Band density/Area
Renal	200
Liver	39
Heart	71
White	105
Brown	21

Figure 6

A.

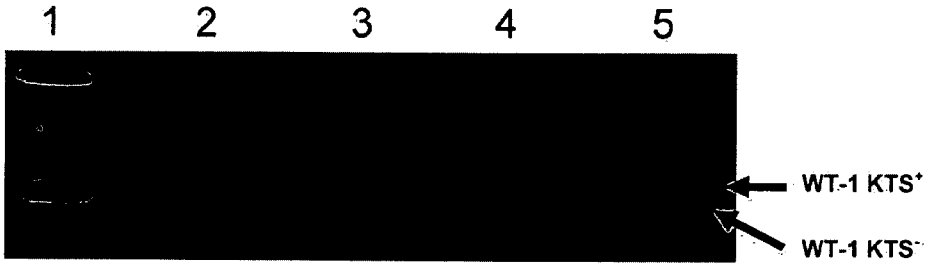
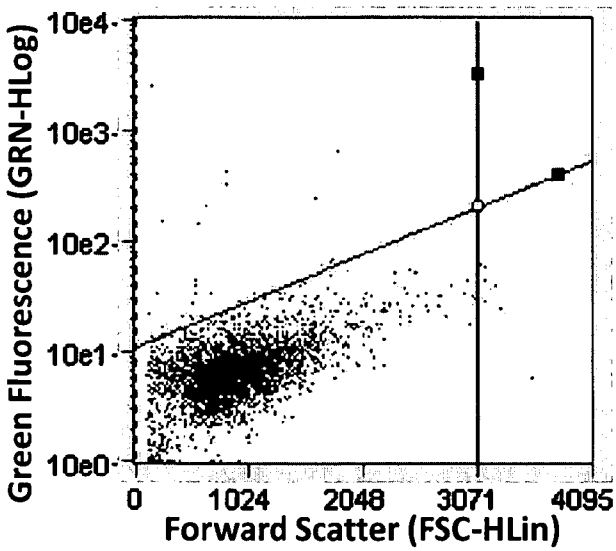


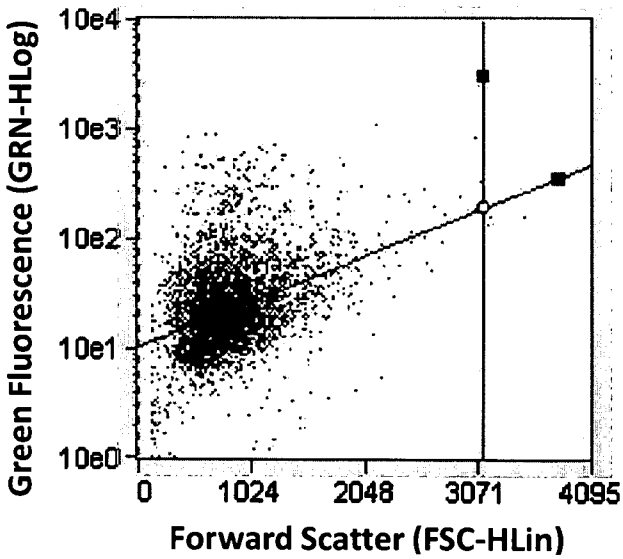
Figure 6

B.



Analysis Results Plot 2 - DotPlot: FSC-HLin vs GRN-HLog

	Count	Cells/mL	% of Gated	x-Mean	y-Mean
LL	3761	6.18e04	99.03%	925.22	7.42
LR	7	1.15e02	0.18%	3246.99	36.67
UL	30	4.93e02	0.79%	682.79	170.28
UR	0	0.00e00	0.00%	0.00	0.00
Gated on Plot 1	3798		75.96%	(% of All Events)	

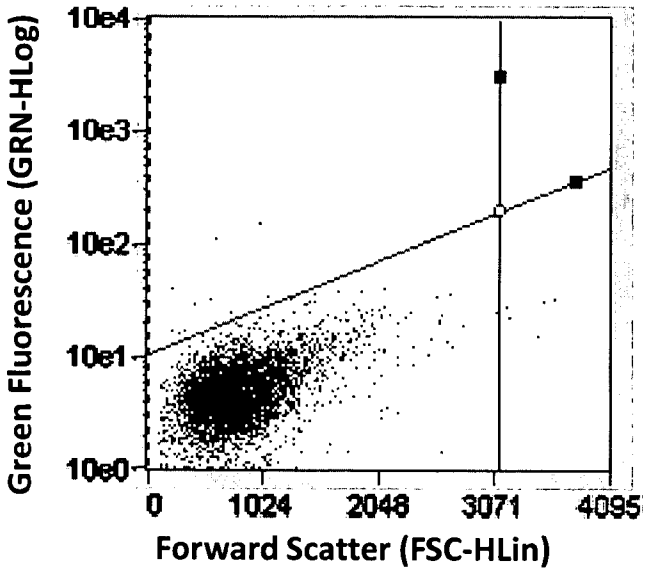


Analysis Results Plot 2 - DotPlot: FSC-HLin vs GRN-HLog

	Count	Cells/mL	% of Gated	x-Mean	y-Mean
LL	1794	2.60e04	45.64%	924.10	17.20
LR	2	2.90e01	0.05%	3395.67	94.32
UL	2130	3.09e04	54.18%	863.96	74.20
UR	5	7.26e01	0.13%	3424.96	591.82
Gated on Plot 1	3931		78.62%	(% of All Events)	

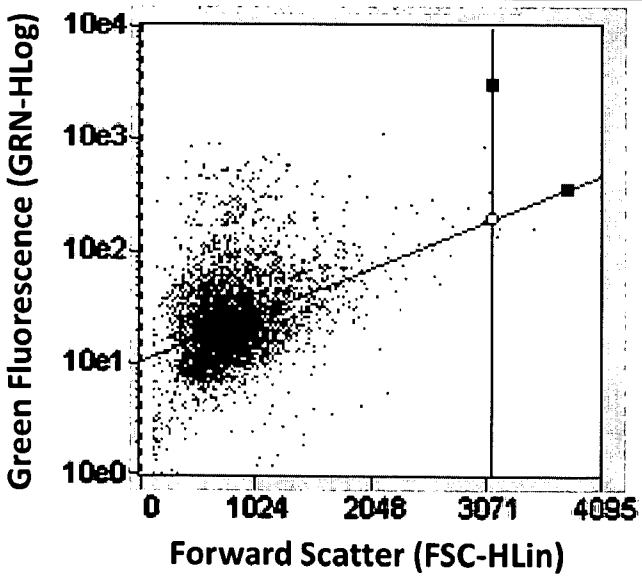
Figure 6

C.



Analysis Results Plot 2 - DotPlot: FSC-HLin vs GRN-HLog

	Count	Cells/mL	% of Gated	x-Mean	y-Mean
LL	4608	2.24e05	99.63%	799.42	5.39
LR	5	2.43e02	0.11%	3411.98	25.43
UL	12	5.83e02	0.26%	591.87	42.26
UR	0	0.00e00	0.00%	0.00	0.00
Gated on Plot 1	4625		92.50%	(% of All Events)	

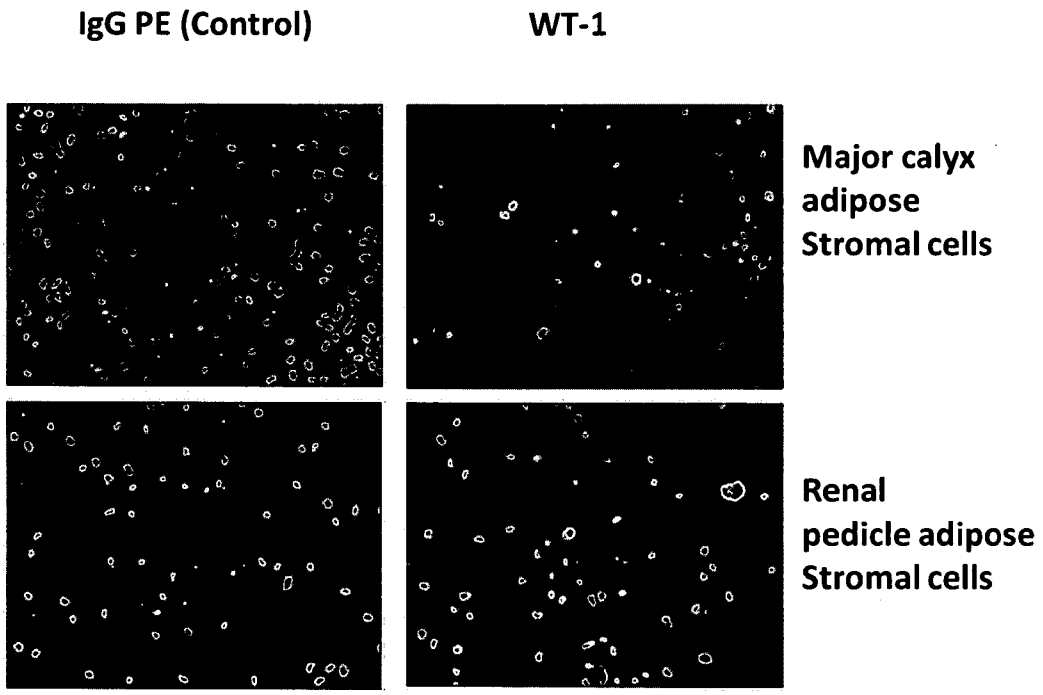


Analysis Results Plot 2 - DotPlot: FSC-HLin vs GRN-HLog

	Count	Cells/mL	% of Gated	x-Mean	y-Mean
LL	2368	7.63e04	52.40%	817.67	14.83
LR	3	9.67e01	0.07%	3300.77	149.33
UL	2148	6.92e04	47.53%	808.91	64.77
UR	0	0.00e00	0.00%	0.00	0.00
Gated on Plot 1	4519		90.38%	(% of All Events)	

Figure 6

D.


IgG PE (Control)
WT-1
Major calyx adipose Stromal cells
Renal pedicle adipose Stromal cells

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/039859

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N5/077 A61K35/55 A61K35/23 A61P13/12
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)
C12N A61K

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SINGH A K ET AL: "Stromal cells cultured from omentum express pluripotent markers, produce high amounts of VEGF, and engraft to injured sites", CELL AND TISSUE RESEARCH, vol. 332, no. 1, 15 January 2008 (2008-01-15), pages 81-88, XP019590731, ISSN: 1432-0878	1-25,30, 31
Y	the whole document	28,29, 32,33

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 August 2011	10/08/2011

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	Teyssier, Bertrand

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SINGH A K ET AL: "Omentum facilitates liver regeneration.", WORLD JOURNAL OF GASTROENTEROLOGY, vol. 15, no. 9, 7 March 2009 (2009-03-07), pages 1057-1064, XP55004012, ISSN: 1007-9327 the whole document	28,29, 32,33
X	LITBARG N O ET AL: "Activated omentum becomes rich in factors that promote healing and tissue regeneration", CELL AND TISSUE RESEARCH, vol. 328, no. 3, 14 February 2007 (2007-02-14), pages 487-497, XP019517927, ISSN: 1432-0878, DOI: DOI:10.1007/S00441-006-0356-4 the whole document	1-25
X,P	WO 2010/111443 A2 (LOYOLA UNIVERSITY CHICAGO [US]; IWASHIMA MAKIO [US]; LOVE ROBERT [US];) 30 September 2010 (2010-09-30) the whole document	1-25
X	US 2007/122393 A1 (MCINTOSH KEVIN R [US] ET AL) 31 May 2007 (2007-05-31) paragraph [0178]	1-27
X	WO 2009/120879 A1 (AMS RES CORP [US]; KOULLICK EDOUARD A [US]; SCHROEDER TANIA MARIE [US]) 1 October 2009 (2009-10-01) paragraph [0053] - paragraph [0054]	1-27
A,P	BASU J ET AL: "Functional evaluation of primary renal cell/biomaterial Neo-Kidney Augment prototypes for renal tissue engineering", CELL TRANSPLANTATION, vol. 20, no. 5, 24 March 2011 (2011-03-24) , page 58PP, XP008140158, ISSN: 0963-6897, DOI: DOI:10.3727/096368911X566172 [retrieved on 2011-05-01] cited in the application	1-32
A,P	PRESNELL S C ET AL: "Isolation, characterization, and expansion methods for defined primary renal cell populations from rodent, canine, and human normal and diseased kidneys.", TISSUE ENGINEERING. PART C, METHODS, vol. 17, no. 3, March 2011 (2011-03), pages 261-273, XP9150678, ISSN: 1937-3392 cited in the application	1-32

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2011/039859

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010111443 A2	30-09-2010	US 2011081321 A1	07-04-2011
US 2007122393 A1	31-05-2007	AU 2006270133 A1	25-01-2007
		BR PI0613190 A2	21-12-2010
		CA 2615391 A1	25-01-2007
		CN 101374945 A	25-02-2009
		EP 1910519 A2	16-04-2008
		JP 2009501526 A	22-01-2009
		KR 20080039903 A	07-05-2008
		US 2011158959 A1	30-06-2011
		WO 2007011797 A2	25-01-2007
WO 2009120879 A1	01-10-2009	AU 2009228203 A1	01-10-2009
		US 2011008299 A1	13-01-2011