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(54) Title: ADHERENT CELLS FROM PLACENTA TISSUE AND USE THEREOF IN THERAPY

(57) Abstract: A method of culturing adherent cells from a placenta or adipose tissue is disclosed. The method comprising culturing the adherent cells from the placenta or adipose tissue under 2 dimensional (2D) culturing conditions which allow cell expansion, the conditions comprising continuous passaging of the cells for at least 4 passages.

ADHERENT CELLS FROM PLACENTA TISSUE AND USE THEREOF IN THERAPY

RELATED APPLICATIONS:

This application claims the benefit of priority from Provisional Patent Application 61/202,049 filed January 23, 2009 and Provisional Patent Application 61/136,377 filed September 2, 2008.

The contents of all of the above documents are incorporated by reference as if fully set forth herein.

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to adherent cells of placenta tissue and, more particularly, but not exclusively, to methods of culturing same and using same for treatment.

In recent years, considerable activity has focused on the therapeutic potential of mesenchymal stromal cells (MSCs) for various medical applications including tissue repair of damaged organs such as the brain, heart, bone and liver and in support of bone marrow transplantations (BMT). MSCs, a heterogeneous population of cells obtained from e.g. bone marrow, adipose tissue, placenta, and blood, is capable of differentiating into different types of cells (e.g. reticular endothelial cells, fibroblasts, adipocytes, osteogenic precursor cells) depending upon influences from various bioactive factors. Accordingly, MSCs have been widely studied in regenerative medicine as the foundation to build new tissues such as bone, cartilage and fat for the repair of injury or replacement of pathologic tissues and as treatment for genetic and acquired diseases [Fibbe and Noort, Ann N Y Acad Sci (2003) 996: 235-44; Horwitz et al., Cytotherapy (2005) 7(5): 393-5; Zimmet and Hare, Basic Res Cardiol (2005) 100(6): 471-81]. Furthermore, the multipotent ability of MSCs, their easy isolation and culture, as well as their high *ex vivo* expansion potential make them an attractive therapeutic tool [Fibbe and Noort, supra; Minguell et al. Exp Biol Med (Maywood) (2001) 226(6): 507-20].

An emerging body of data indicates that MSCs escape recognition of alloreactive cells and are considered to be immune privileged [Le Blanc et al., Exp Hematol (2003) 31(10): 890-6]. Having low immunogenicity, MSCs are not rejected by the patient's immune system and therefore are considered not to require HLA matching.

Placental derived MSCs exhibit many markers common to MSCs isolated from other tissues, e.g. CD105, CD73, CD90 and CD29, and the lack of expression of hematopoietic, endothelial and trophoblastic-specific cell markers. Adipogenic, osteogenic, and neurogenic differentiation have been achieved after culturing placental derived MSCs under appropriate conditions [Yen et al., Stem Cells (2005) 23(1): 3-9]. Furthermore, MSCs isolated from placenta and cultured *in vitro* have been demonstrated to be immune privileged in a similar fashion as MSCs [Li et al., Cell Res (2005) 15(7): 539-47]. Thus, the placenta provides an ethically non-controversial and easily accessible source of MSCs for experimental and clinical applications [Zhang et al., Exp Hematol (2004) 32(7): 657-64]. In addition, the present inventors have previously devised three dimensional (3D) culturing conditions suitable for expansion of placental derived MSCs (WO/2007/108003).

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a method of culturing adherent cells from a placenta or adipose tissue, the method comprising culturing the adherent cells from the placenta or adipose tissue under 2 dimensional (2D) culturing conditions which allow cell expansion, the conditions comprising continuous passaging of the cells for at least 4 passages.

According to some embodiments of the invention, the method further comprises growing the cells in a culture medium devoid of an antibiotic from at least passage 2.

According to an aspect of some embodiments of the present invention there is provided a population of cells generated according to the above method.

According to an aspect of some embodiments of the present invention there is provided a population of cells comprising a gene expression profile essentially as described herein.

According to an aspect of some embodiments of the present invention there is provided a use of the population of cells, for the manufacture of a medicament identified for treating a condition which can benefit from cell or organ transplantation.

According to an aspect of some embodiments of the present invention there is provided a method of inducing tolerance and/or immunosuppression in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of adherent cells, thereby inducing tolerance and/or immunosuppression in the subject.

According to some embodiments of the invention, at least 12 % of the adherent cells are at a proliferative phase.

According to some embodiments of the invention, the adherent cells are capable of suppressing an immune reaction.

According to some embodiments of the invention, the adherent cells comprise a positive marker expression selected from the group consisting of CD73, CD90, CD29 and CD105.

According to some embodiments of the invention, the adherent cells comprise a negative marker expression selected from the group consisting of CD11b, CD34, HLA-DR, CD14, CD19, CD200 and CD45.

According to some embodiments of the invention, the adherent cells comprise a gene expression profile essentially as described herein.

According to some embodiments of the invention, the adherent cells are less committed to an osteogenic lineage as compared to adherent cells from bone marrow grown and allowed to differentiate under the same conditions.

According to some embodiments of the invention, the adherent cells are less committed to an adipogenic lineage as compared to adherent cells from bone marrow grown and allowed to differentiate under the same conditions.

According to some embodiments of the invention, the condition is selected from the group consisting of ischemia, peripheral arterial disease (PAD), critical limb ischemia (CLI), lower extremity ischemia, ischemic vascular disease, vascular disease of the kidney, ischemic heart disease, myocardial ischemia, coronary artery disease (CAD), atherosclerotic cardiovascular disease, left main coronary artery disease, arterial occlusive disease, peripheral ischemia, peripheral vascular disease, arteriosclerosis,

ischemic brain disease, stroke, cerebral ischemia, cerebro vascular disease, retinopathy, retinal repair, remodeling disorder, von Hippel-Lindau syndrome, hereditary hemorrhagic telengiectasiaischemic vascular disease, Buerger's disease, ischemic renal disease, ischemic placenta, reproduction associated disorders, graft-versus-host disease, solid organ transplantation, hematopoietic stem cell transplantation, diabetes, connective tissue damage, cancer, pre-cancer, bone cancer, osteosarcoma, bone metastases, bone fracture, burn wound, articular cartilage defect, deep wound, delayed wound-healing, delayed ulcer healing, subchondral-bone cyst, osteoporosis, osteoarthritis, degenerated bone, cartilage damage, articular cartilage defect, injured tendons, autoimmune diseases, metabolic disorders, psoriasis, neuropathic pain, peripheral nerve injury, support of kidney transplantation and inflammatory diseases.

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

BRIEF DESCRIPTION OF THE DRAWINGS

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

In the drawings:

FIGs. 1A-B are figures depicting cell cycle analysis of adherent cells of placenta grown according to the present teachings (Figure 1A) or adherent cells manufactured according to the teachings of WO/2007/108003, designated PLX (Figure 1B). Cells were fixed in 70 % EtOH O.N, centrifuged and re-suspended in a Propidium Iodide (PI) solution and then analyzed by FACS.

FIG. 2 is a bar graph depicting marker expression on adherent cells of placenta grown according to the present teachings. Of note, negative expression was recorded for CD11b, CD34, HLA-DR, CD14, CD19 CD200 and CD45, while positive expression was noted for CD29, CD73, CD90 and CD105.

FIG. 3 is a bar graph depicting reduction of lymphocyte cell response by adherent cells of placenta grown according to the present teachings. Peripheral blood (PB) derived mononuclear cells (MNCs) were stimulated with PHA (10 μg/ml). One of four different batches of 2D adherent cells were added to the stimulated MNCs. Three replicates of each group were seeded in 96-well plates.

FIGs. 4A-F are photographs depicting growth of bone marrow and placenta cells under osteogenesis or adipogenesis differentiation conditions. Bone marrow derived cells (Figures 4A-C) or placenta derived cells (Figures 4D-F) were plated in growth medium (Figures 4A and 4D), osteogenesis differentiation medium (Figures 4B and 4E) or adipogenesis differentiation medium (Figures 4C and 4F) in a 24 well plate coated with vitronectin and collagen. Medium was replaced every 3-4 days. At the end of growth period cells were fixed, stained and pictured as described in detail the Examples section which follows.

FIGs. 5A-F are photographs depicting growth of bone marrow and placenta cells under modified osteogenesis or adipogenesis differentiation conditions. Bone marrow derived cells (Figures 5A-C) or placenta derived cells (Figures 5D-F) were plated in growth medium (Figures 5A and 5D), osteogenesis differentiation medium (Figures 5B and 5E) or adipogenesis differentiation medium (Figures 5C and 5F) in a 24 well plate coated with vitronectin and collagen. Medium was replaced every 3-4 days. At the end of growth period cells were fixed, stained and pictured as described in detail the Examples section which follows.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to adherent cells of placenta tissue and, more particularly, but not exclusively, to methods of culturing same and using same for treatment.

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

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

While reducing the present invention to practice, the present inventors have uncovered that placenta adherent cells cultured ex vivo under specific 2D conditions, are capable of suppressing an immune response and are highly proliferative and thus may be used for therapeutic applications.

As is illustrated herein below and in Example 1-3 of the Examples section which follows, the present inventors were able to expand placenta-derived adherent cells under specific 2D conditions. The 2D conditions of the present invention comprise continuous passaging of the cells for at least 4 passages and optionally include incubating the cells with antibiotic supplement only during the initial two passages of cell growth (see Example 2). Moreover, unlike the previously described methods (see Example 1 and WO/2007/108003) wherein the cells are cryopreserved during the growth process, the present teachings teach cryopreservation of the cells only at the end of culture (e.g., after 5-6 passages). As is shown in Example 3, the placenta derived 2D adherent cells of the present invention comprise stromal stem cells properties e.g. they express cellular markers typical of stromal stem cells and comprise immunosuppressive properties. Furthermore, these cells are highly proliferative (28 % of cells were in S and G2/M phases) suggesting that the 2D culturing conditions are optimal to support cell growth.

In addition, the placenta derived 2D adherent cells of the present invention did not differentiation into osteocytes (Examples 4-5) or adipocytes (Examples 6-7), in sharp contrast to bone marrow adherent cells grown under the same conditions.

Thus, according to one aspect of the present invention there is provided a method of culturing adherent cells from a placenta or adipose tissue, the method comprising culturing the adherent cells from the placenta or adipose tissue under 2 dimensional (2D) culturing conditions which allow cell expansion, the conditions comprising continuous passaging of the cells for at least 4 passages.

As used herein the phrase "adherent cells" refers to a homogeneous or heterogeneous population of cells which are anchorage dependent, i.e., require attachment to a surface in order to grow *in vitro*.

As used herein the phrase "adipose tissue" refers to a connective tissue which comprises fat cells (adipocytes).

As used herein the term "placenta tissue" refers to any portion of the mammalian organ which lines the uterine wall and during pregnancy envelopes the fetus, to which it is attached by the umbilical cord. Following birth, the placenta is expelled (and is referred to as a post partum placenta). In an exemplary embodiment, placenta refers to whole placenta.

According to the present teachings, placenta or adipose tissue derived adherent cells are propagated using two dimensional (2D) culturing conditions.

As used herein the phrase "two dimensional culture" refers to a culture in which the cells are disposed to conditions which are compatible with cell growth while allowing the cells to grow in one plane. The conditions in the two dimensional culture of the invention are designed to enable expansion of the adherent cells.

As used herein the terms "expanding" and "expansion" refer to substantially differentiation-less maintenance of the cells and ultimately cell growth, i.e., increase of a cell population (e.g., at least 2 fold) without differentiation accompanying such increase.

As used herein the terms "maintaining" and "maintenance" refer to substantially differentiation-less cell renewal, i.e., substantially stationary cell population without differentiation accompanying such stationarity.

As mentioned, the adherent cells of this aspect of the invention are retrieved from a placental or adipose tissue.

Placental cells may be obtained from a full-term or pre-term placenta. Placenta is preferably collected once it has been ex blooded. The placenta is preferably perfused for a period of time sufficient to remove residual cells (i.e., red blood cells). The term "perfuse" or "perfusion" used herein refers to the act of pouring or passaging a fluid over the placenta or in the later stages of the method over the cultured cells. The placental tissue may be from any mammal; for example, the placental tissue is human. A convenient source of placental tissue is from a post partum placenta (e.g., 1-6 hours),

however, the source of placental tissue or cells or the method of isolation of placental tissue is not critical to the invention.

Placenta derived adherent cells may be obtained from both fetal (*i.e.*, amnion, chorion, chorionic villi or inner parts of the placenta, see Example 1) and maternal (*i.e.*, decidua basalis, and decidua parietalis) parts of the placenta. Tissue specimens are washed in a physiological buffer [e.g., phosphate-buffered saline (PBS) or Hank's buffer]. Single-cell suspensions are made by treating the tissue with a digestive enzyme (see below) or/and mincing and flushing the tissue parts through a nylon filter or by gentle pipetting (Falcon, Becton, Dickinson, San Jose, CA) with washing medium.

It will be appreciated that adherent cells may be derived from adipose tissue. Adipose tissue derived adherent cells may be isolated by a variety of methods known to those skilled in the art. For example, such methods are described in U.S. Pat. No. 6,153,432. The adipose tissue may be derived from omental/visceral, mammary, gonadal, or other adipose tissue sites. One source of adipose tissue is omental adipose. In humans, the adipose is typically isolated by liposuction.

Isolated adherent cells from placenta or adipose tissue may be derived by treating the tissue with a digestive enzyme such as collagenase, trypsin and/or dispase; and/or effective concentrations of hyaluronidase or DNAse; and ethylenediaminetetra-acetic acid (EDTA); at temperatures between 25 – 50 °C, for periods of between 10 minutes to 3 hours. The cells may then be passed through a nylon or cheesecloth mesh filter of between 20 microns to 1 mm. Cells are centrifuged at speeds of between 100 to 3000 x g for periods of between 1 minutes to 1 hour at temperatures of between 4- 50 °C (see U.S. Pat. No. 7,078,230).

Cell retrieval from placenta or adipose tissue is preferably effected under aseptic conditions. Once isolated cells are obtained, they are allowed to adhere to an adherent material (e.g., configured as a surface) to thereby isolate adherent cells. Culturing then proceeds under 2D conditions (as described herein and in Example 2 of the Examples section which follows).

As used herein "an adherent material" refers to a synthetic, naturally occurring or a combination of same of a non-cytotoxic (i.e., biologically compatible) material having a chemical structure (e.g., charged surface exposed groups) which may retain the cells on a surface.

Examples of adherent materials which may be used in accordance with this aspect of the invention include, but are not limited to, a polyester, a polypropylene, a polyalkylene, a polyfluorochloroethylene, a polyvinyl chloride, a polystyrene, a polysulfone, a cellulose acetate, a glass fiber, a ceramic particle, a matrigel, an extra cellular matrix component (e.g., fibronectin, vitronectin chondronectin, laminin), a dextran, a collagen, a poly L lactic acid and an inert metal fiber.

It will be appreciated that seeding of placenta or adipose cells is typically effected at a culture density of $3 \pm 0.2 \times 10^3$ cells/cm^2. Following seeding, cell cultures are usually cultured in a tissue culture incubator under humidified conditions with 5 % CO2 at 37 °C.

Further steps of purification or enrichment for stromal stem cells may be effected using methods which are well known in the art (such as by FACS using stromal stem cell marker expression, as further described herein below).

Non-limiting examples of base media useful in culturing according to the invention include Minimum Essential Medium Eagle, ADC-1, LPM (Bovine Serum Albumin-free), F10 (HAM), F12 (HAM), DCCM1, DCCM2, RPMI 1640, BGJ Medium (with and without Fitton-Jackson Modification), Basal Medium Eagle (BME-with the addition of Earle's salt base), Dulbecco's Modified Eagle Medium (DMEM-without serum), Yamane, IMEM-20, Glasgow Modification Eagle Medium (GMEM), Leibovitz L-15 Medium, McCoy's 5A Medium, Medium M199 (M199E-with Earle's sale base), Medium M199 (M199H-with Hank's salt base), Minimum Essential Medium Eagle (MEM-E-with Earle's salt base), Minimum Essential Medium Eagle (MEM-H-with Hank's salt base) and Minimum Essential Medium Eagle (MEM-NAA with non essential amino acids), among numerous others, including medium 199, CMRL 1415, CMRL 1969, CMRL 1066, NCTC 135, MB 75261, MAB 8713, DM 145, Williams' G, Neuman & Tytell, Higuchi, MCDB 301, MCDB 202, MCDB 501, MCDB 401, MCDB 411, MDBC 153. A preferred medium for use in the invention is DMEM. These and other useful media are available from GIBCO, Grand Island, N.Y., USA and Biological Industries, Bet HaEmek, Israel, among others. A number of these media are summarized in Methods in Enzymology, Volume LVIII, "Cell Culture", pp. 62 72, edited by William B. Jakoby and Ira H. Pastan, published by Academic Press, Inc.

The medium may be supplemented such as with serum such as fetal serum of human, bovine or other species, and optionally or alternatively, growth factors, vitamins (e.g. ascorbic acid), cytokines, salts (e.g. B-glycerophosphate), steroids (e.g. dexamethasone) and hormones e.g., growth hormone, erythropoeitin, thrombopoietin, interleukin 3, interleukin 6, interleukin 7, macrophage colony stimulating factor, c-kit ligand/stem cell factor, osteoprotegerin ligand, insulin, insulin like growth factors, epidermal growth factor, fibroblast growth factor, nerve growth factor, cilary neurotrophic factor, platelet derived growth factor, and bone morphogenetic protein at concentrations of between picogram/ml to milligram/ml levels.

It is further recognized that additional components may be added to the culture medium. Such components may be antibiotics, antimycotics, albumin, amino acids, and other components known to the art for the culture of cells. Additionally, components may be added to enhance the differentiation process when needed (see further below).

According to an embodiment of the present invention, the cells are grown in a culture medium devoid of antibiotic supplements from at least passage 2, at least passage 3, or at least passage 4.

As mentioned, once adherent cells are at hand they may be continuously passaged. According to an embodiment of the present invention, the cells are passaged for at least 4 passages, at least 5 passages, at least 6 passages, at least 7 passages or at least 8 passages. It will be appreciated that cells are typically passaged when the culture reaches about 70-90 % confluence, typically after 3-7 days (e.g., 3-5 days, 1-3 doublings).

Culturing is effected for at least about 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 14 days, 20 days, a month or even more. Passaging may also be effected to increase cell number. It will be appreciated that culture medium may be changed in order to prolong and improve culturing conditions.

Cells can be harvested when at least about 10 % of cells are proliferating while avoiding uncontrolled differentiation and senescence.

Adherent cells of some embodiments of the present invention comprise at least about 10 %, 28 %, 30 %, 50 %, 80 % or more proliferative cells (as can be assayed by FACS monitoring S and G2/M phases).

Adherent cells of some embodiments of the invention may comprise at least one "stromal stem cell phenotype".

As used herein "a stromal stem cell phenotype" refers to a structural or functional phenotype typical of a bone-marrow derived stromal (i.e., mesenchymal) stem cell.

As used herein the phrase "stem cell" refers to a cell which is not terminally differentiated.

Thus for example, the cells may have a spindle shape. Alternatively or additionally the cells may express a marker or a collection of markers (e.g. surface marker) typical to stromal stem cells. Examples of stromal stem cell surface markers (positive and negative) include but are not limited to CD105+, CD29+, CD44+, CD73+, CD90+, CD3-, CD4-, CD34-, CD45-, CD80-, CD5-, CD20-, CD11B-, CD14-, CD19-, CD79-, HLA-DR-, and FMC7-. Other stromal stem cell markers include but are not limited to tyrosine hydroxylase, nestin and H-NF.

Adherent cells of placenta tissue generated according to the present teachings have a gene expression profile essentially as described in Example 3 of the Examples section which follows.

Examples of functional phenotypes typical of stromal stem cells include at least one of the following, but are not limited to, T cell suppression activity (they don't stimulate T cells and conversely suppress same) and hematopoietic stem cell support activity.

According to an exemplary embodiment, the adherent cells of the present invention are less committed to differentiation into osteogenic or adipogenic lineages as compared to adherent cells from the bone marrow grown and differentiated under the same conditions (see Examples 4-5 and Examples 6-7, respectively).

As is shown in Example 3 of the Examples section which follows, the adherent 2D cells of the present invention were found to suppress the immune reaction of human peripheral blood mononuclear cells, thus the cells exhibit biological activities which may be preferentially used in the clinic (e.g., T cell suppression activity, hematopoietic stem cell support activity).

According to one embodiment of the invention, the adherent cells of the invention are capable of suppressing an immune reaction in a subject.

As used herein the phrase "suppressing an immune reaction in a subject" refers to decreasing or inhibiting the immune reaction occurring in a subject in response to an antigen (e.g., a foreign cell or a portion thereof). The immune response which can be suppressed by the adherent cells include the humoral immune responses, and cellular immune responses, which involve specific recognition of pathogen antigens via antibodies and T-lymphocytes (proliferation of T cells), respectively.

The populations of cells generated according to the present teachings may be used for treating a condition which can benefit from cell or organ transplantation.

As used herein, the term "condition" refers to any pathology (disease, condition, syndrome or disorder) which may benefit from cell (e.g. stem cell) or organ transplantation. Examples include ischemic conditions, cardiovascular conditions, nervous system conditions, gastrointestinal tract conditions, orthopedic conditions, hematopoietic conditions, renal conditions and hepatic conditions, such as but are not limited to, peripheral arterial disease (PAD), such as limb ischemia and critical limb ischemia (CLI), lower extremity ischemia, ischemic vascular disease, ischemic heart disease, myocardial ischemia, acute myocardial infarction (MI), coronary artery disease (CAD), atherosclerotic cardiovascular disease, left main coronary artery disease, arterial occlusive disease, peripheral ischemia, peripheral vascular disease, arteriosclerosis, ischemic brain disease, stroke, cerebral ischemia, cerebro vascular disease, retinopathy, retinal repair, remodeling disorder, von Hippel-Lindau syndrome, hereditary hemorrhagic telengiectasiaischemic vascular disease, Buerger's disease, diabetes, vascular disease of the kidney, ischemic renal disease, liver disease, ischemic placenta, reproduction associated disorders, graft-versus-host disease (GVHD), solid organ transplant, hematopoietic stem cell transplantation (HSCT), inflammatory conditions of the gastrointestinal (GI) tract, ulcerative colitis, delayed wound-healing, delayed ulcer healing, cancer (e.g. breast cancer), pre-cancer, conditions characterized by connective tissue damage such as bone cancer, osteosarcoma, bone metastases, bone fracture, degenerative disc disease, osteogenesis imperfecta (OI), burn, burn wound, articular cartilage defect, deep wound, delayed wound-healing, delayed ulcer healing, metabolic disorders, psoriasis, neuropathic pain, peripheral nerve injury, support of kidney transplantation, subchondral-bone cyst, osteoporosis, osteoarthritis (OA), degenerated

bone, cartilage damage, articular cartilage defect, injured tendons (e.g. overstrain-induced injuries of tendons) and injured ligaments.

It will be appreciated that the adherent cells of the present invention are capable of inducing immunosuppression and/or tolerance in a subject. Thus, the adherent cells may be used to treat any condition in need of immunosuppression and/or tolerance. Such conditions included, but are not limited to, autoimmune diseases and inflammatory diseases (including acute and chronic inflammatory diseases) including, but are not limited to, cardiovascular diseases, rheumatoid diseases, glandular diseases, gastrointestinal diseases, cutaneous diseases, hepatic diseases, neurological diseases, muscular diseases, nephric diseases, diseases related to reproduction, connective tissue diseases and systemic diseases.

Examples of autoimmune cardiovascular diseases include, but are not limited to atherosclerosis (Matsuura E. *et al.,* Lupus. 1998;7 Suppl 2:S135), myocardial infarction (Vaarala O. Lupus. 1998;7 Suppl 2:S132), thrombosis (Tincani A. *et al.,* Lupus 1998;7 Suppl 2:S107-9), Wegener's granulomatosis, Takayasu's arteritis, Kawasaki syndrome (Praprotnik S. *et al.,* Wien Klin Wochenschr 2000 Aug 25;112 (15-16):660), anti-factor VIII autoimmune disease (Lacroix-Desmazes S. *et al.,* Semin Thromb Hemost.2000;26 (2):157), necrotizing small vessel vasculitis, microscopic polyangiitis, Churg and Strauss syndrome, pauci-immune focal necrotizing and crescentic glomerulonephritis (Noel LH. Ann Med Interne (Paris). 2000 May;151 (3):178), antiphospholipid syndrome (Flamholz R. *et al.,* J Clin Apheresis 1999;14 (4):171), antibody-induced heart failure (Wallukat G. *et al.,* Am J Cardiol. 1999 Jun 17;83 (12A):75H), thrombocytopenic purpura (Moccia F. Ann Ital Med Int. 1999 Apr-Jun;14 (2):114; Semple JW. *et al.,* Blood 1996 May 15;87 (10):4245), autoimmune hemolytic anemia (Efremov DG. *et al.,* Leuk Lymphoma 1998 Jan;28 (3-4):285; Sallah S. *et al.,* Ann Hematol 1997 Mar;74 (3):139), cardiac autoimmunity in Chagas' disease (Cunha-Neto E. *et al.,* J Clin Invest 1996 Oct 15;98 (8):1709) and anti-helper T lymphocyte autoimmunity (Caporossi AP. *et al.,* Viral Immunol 1998;11 (1):9).

Examples of autoimmune rheumatoid diseases include, but are not limited to rheumatoid arthritis (Krenn V. *et al.,* Histol Histopathol 2000 Jul;15 (3):791; Tisch R, McDevitt HO. Proc Natl Acad Sci units S A 1994 Jan 18;91 (2):437) and ankylosing spondylitis (Jan Voswinkel *et al.,* Arthritis Res 2001; 3 (3): 189).

Examples of autoimmune glandular diseases include, but are not limited to, pancreatic disease, Type I diabetes, thyroid disease, Graves' disease, thyroiditis, spontaneous autoimmune thyroiditis, Hashimoto's thyroiditis, idiopathic myxedema, ovarian autoimmunity, autoimmune anti-sperm infertility, autoimmune prostatitis and Type I autoimmune polyglandular syndrome. diseases include, but are not limited to autoimmune diseases of the pancreas, Type 1 diabetes (Castano L. and Eisenbarth GS. Ann. Rev. Immunol. 8:647; Zimmet P. Diabetes Res Clin Pract 1996 Oct;34 Suppl:S125), autoimmune thyroid diseases, Graves' disease (Orgiazzi J. Endocrinol Metab Clin North Am 2000 Jun;29 (2):339; Sakata S. *et al.,* Mol Cell Endocrinol 1993 Mar;92 (1):77), spontaneous autoimmune thyroiditis (Braley-Mullen H. and Yu S, J Immunol 2000 Dec 15;165 (12):7262), Hashimoto's thyroiditis (Toyoda N. *et al.,* Nippon Rinsho 1999 Aug;57 (8):1810), idiopathic myxedema (Mitsuma T. Nippon Rinsho. 1999 Aug;57 (8):1759), ovarian autoimmunity (Garza KM. *et al.,* J Reprod Immunol 1998 Feb;37 (2):87), autoimmune anti-sperm infertility (Diekman AB. *et al.,* Am J Reprod Immunol. 2000 Mar;43 (3):134), autoimmune prostatitis (Alexander RB. *et al.,* Urology 1997 Dec;50 (6):893) and Type I autoimmune polyglandular syndrome (Hara T. *et al.,* Blood. 1991 Mar 1;77 (5):1127).

Examples of autoimmune gastrointestinal diseases include, but are not limited to, chronic inflammatory intestinal diseases (Garcia Herola A. *et al.,* Gastroenterol Hepatol. 2000 Jan;23 (1):16), celiac disease (Landau YE. and Shoenfeld Y. Harefuah 2000 Jan 16;138 (2):122), colitis, ileitis and Crohn's disease.

Examples of autoimmune cutaneous diseases include, but are not limited to, autoimmune bullous skin diseases, such as, but are not limited to, pemphigus vulgaris, bullous pemphigoid and pemphigus foliaceus.

Examples of autoimmune hepatic diseases include, but are not limited to, hepatitis, autoimmune chronic active hepatitis (Franco A. *et al.,* Clin Immunol Immunopathol 1990 Mar;54 (3):382), primary biliary cirrhosis (Jones DE. Clin Sci (Colch) 1996 Nov;91 (5):551; Strassburg CP. *et al.,* Eur J Gastroenterol Hepatol. 1999 Jun;11 (6):595) and autoimmune hepatitis (Manns MP. J Hepatol 2000 Aug;33 (2):326).

Examples of autoimmune neurological diseases include, but are not limited to, multiple sclerosis (Cross AH. *et al.,* J Neuroimmunol 2001 Jan 1;112 (1-2):1),

Alzheimer's disease (Oron L. *et al.,* J Neural Transm Suppl. 1997;49:77), myasthenia gravis (Infante AJ. And Kraig E, Int Rev Immunol 1999;18 (1-2):83; Oshima M. *et al.,* Eur J Immunol 1990 Dec;20 (12):2563), neuropathies, motor neuropathies (Kornberg AJ. J Clin Neurosci. 2000 May;7 (3):191); Guillain-Barre syndrome and autoimmune neuropathies (Kusunoki S. Am J Med Sci. 2000 Apr;319 (4):234), myasthenia, Lambert-Eaton myasthenic syndrome (Takamori M. Am J Med Sci. 2000 Apr;319 (4):204); paraneoplastic neurological diseases, cerebellar atrophy, paraneoplastic cerebellar atrophy and stiff-man syndrome (Hiemstra HS. *et al.,* Proc Natl Acad Sci units S A 2001 Mar 27;98 (7):3988); non-paraneoplastic stiff man syndrome, progressive cerebellar atrophies, encephalitis, Rasmussen's encephalitis, amyotrophic lateral sclerosis, Sydeham chorea, Gilles de la Tourette syndrome and autoimmune polyendocrinopathies (Antoine JC. and Honnorat J. Rev Neurol (Paris) 2000 Jan;156 (1):23); dysimmune neuropathies (Nobile-Orazio E. *et al.,* Electroencephalogr Clin Neurophysiol Suppl 1999;50:419); acquired neuromyotonia, arthrogryposis multiplex congenita (Vincent A. *et al.,* Ann N Y Acad Sci. 1998 May 13;841:482), neuritis, optic neuritis (Soderstrom M. *et al.,* J Neurol Neurosurg Psychiatry 1994 May;57 (5):544) and neurodegenerative diseases.

Examples of autoimmune muscular diseases include, but are not limited to, myositis, autoimmune myositis and primary Sjogren's syndrome (Feist E. *et al.,* Int Arch Allergy Immunol 2000 Sep;123 (1):92) and smooth muscle autoimmune disease (Zauli D. *et al.,* Biomed Pharmacother 1999 Jun;53 (5-6):234).

Examples of autoimmune nephric diseases include, but are not limited to, nephritis and autoimmune interstitial nephritis (Kelly CJ. J Am Soc Nephrol 1990 Aug;1 (2):140).

Examples of autoimmune diseases related to reproduction include, but are not limited to, repeated fetal loss (Tincani A. *et al.,* Lupus 1998;7 Suppl 2:S107-9).

Examples of autoimmune connective tissue diseases include, but are not limited to, ear diseases, autoimmune ear diseases (Yoo TJ. *et al.*, Cell Immunol 1994 Aug;157 (1):249) and autoimmune diseases of the inner ear (Gloddek B. *et al.*, Ann N Y Acad Sci 1997 Dec 29;830:266).

Examples of autoimmune systemic diseases include, but are not limited to, systemic lupus erythematosus (Erikson J. *et al.,* Immunol Res 1998;17 (1-2):49) and

systemic sclerosis (Renaudineau Y. *et al.,* Clin Diagn Lab Immunol. 1999 Mar;6 (2):156); Chan OT. *et al.,* Immunol Rev 1999 Jun;169:107).

Furthermore, the adherent cells may be used to treat diseases associated with transplantation of a graft including, but are not limited to, graft rejection, chronic graft rejection, subacute graft rejection, hyperacute graft rejection, acute graft rejection and graft versus host disease.

As used herein the term "treating" refers to inhibiting or arresting the development of a pathology and/or causing the reduction, remission, or regression of a pathology. Those of skill in the art will understand that various methodologies and assays can be used to assess the development of a pathology, and similarly, various methodologies and assays may be used to assess the reduction, remission or regression of a pathology. The term "treating" may also refer to alleviating or diminishing a symptom associated with the pathology.

The subject treated by the adherent cells may be any subject (e.g., a mammal), such as a human subject or a domesticated animal including, but not limited to, horses (i.e. equine), cattle, goat, sheep, pig, dog, cat, camel, alpaca, llama and yak who is diagnosed with or suffers from the pathology and can benefit from stem cell transplantation.

Methods of deriving lineage specific cells from the adherent cells (e.g. stromal stem cells) of the invention are well known in the art. See for example, U.S. Pat. Nos. 5,486,359, 5,942,225, 5,736,396, 5,908,784 and 5,902,741.

The adherent cells may be naïve or may be genetically modified such as to derive a lineage of interest (see U.S. Pat. Appl. No. 20030219423).

The cells may be of autologous or non-autologous source (i.e., allogeneic or xenogeneic) of fresh or frozen (e.g., cryo-preserved) preparations.

Depending on the medical condition, the subject may be administered with additional chemical drugs (e.g., immunomodulatory, chemotherapy etc.) or cells.

Even though the cells are characterized by immuno-suppressive activity, they may still provoke host or donor-derived undesirable immune response. Approaches have been developed to reduce the likelihood of rejection of non-autologous cells. These include either suppressing the recipient immune system or encapsulating the non-autologous cells in immunoisolating, semipermeable membranes before transplantation.

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

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

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

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

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

medical devices. Med Device Technol. 1999, 10: 6-9; Desai, T.A. Microfabrication technology for pancreatic cell encapsulation. Expert Opin Biol Ther. 2002, 2: 633-46).

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

Furthermore, it will be appreciated that the cells can be administered either *per se* or, preferably as a part of a pharmaceutical composition that further comprises a pharmaceutically acceptable carrier.

As used herein a "pharmaceutical composition" refers to a preparation of the adherent cells of the invention (i.e., adherent cells), with other chemical components such as pharmaceutically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of the cells to a subject.

Hereinafter, the term "pharmaceutically acceptable carrier" refers to a carrier or a diluent that does not cause significant irritation to a subject and does not abrogate the biological activity and properties of the administered compound. Examples, without limitations, of carriers are propylene glycol, saline, emulsions and mixtures of organic solvents with water.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

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

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

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, physiological salt buffer, or freezing medium containing cryopreservents.

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

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures *in vitro*, in cell cultures or experimental animals.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations. However, the amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Following transplantation, a portion of the cells of the invention preferably survive in the diseased area for a period of time (e.g. about 2-6 weeks), such that a therapeutic effect is observed.

Compositions including the preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

Compositions of the invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be

accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert.

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

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

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

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

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

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

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges

between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

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

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

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

EXAMPLES

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

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific

American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1

Production of placenta derived 3D adherent cells

Adherent cells were produced as was previously described (see WO/2007/108003) in a bioreactor system containing 3D carriers to produce 3D-adherent cells (designated herein as PLX).

Materials and Experimental Procedures

Placenta derived adherent cells - Inner parts of a full-term delivery placenta (Bnei Zion medical center, Haifa, Israel) were cut under aseptic conditions, washed 3

times with Hank's Buffer and incubated for 3 hours at 37 °C with 0.1 % Collagenase (1mg / ml tissue; Sigma- Aldrich, St. Lewis, MO). Using gentle pipetting, suspended cells were then washed with DMEM supplemented with 10 % FCS, Pen-Strep-Nystatin mixture (100 U/ml:100 μg/ml:1.25 un/ml) and 2 mM L-glutamine, seeded in 75 cm^2 flasks and incubated at 37 °C in a tissue culture incubator under humidified condition with 5 % CO_2.

Two dimensional (2D) cell growth

Cells were allowed to adhere to a plastic surface for 48-72 hours after which the media was changed every 3-4 days. After 2-3 passages, the cells were cryopreserved, thawed and seeded for a secondary growth in flasks. When reaching 60-80 % confluence cells were detached from the growth flask using 0.25 % trypsin-EDTA and seeded into new flasks (usually every 3-5 days), for another 2-5 passages. Cultured cells were thereafter collected for analysis or for culturing in bioreactors.

PluriX™ Plug Flow bioreactor - The PluriX™ Plug Flow bioreactor (Pluristem, Haifa, Israel; see U.S. Pat. No. 6,911,201 and WO/2007/108003), was loaded with 1-100 ml packed 3D porrosive carriers (4 mm in diameter) made of a non woven fabric matrix of polyester. These carriers enable the propagation of large cell numbers in a relatively small volume. Glassware was designed and manufactured by Pluristem (Pluristem, Haifa, Israel). The bioreactor was maintained in an incubator of 37 °C, with flow rate regulated and monitored by a valve, and peristaltic pump. The bioreactor contains a sampling and injection point, allowing the sequential seeding of cells. Culture medium was supplied at pH 6.7-7.4 from a reservoir. The reservoir was supplied by a filtered gas mixture, containing air/CO_2/O_2 at differing proportions, depending on cell density in the bioreactor. The O_2 proportion was suited to the level of dissolved O_2 at the bioreactor exit, determined by a monitor. The gas mixture was supplied to the reservoir via silicone tubes or diffuser (Degania Bet, Emek Hayarden, Israel). The culture medium was passed through a separating container which enables collection of circulating, nonadherent cells. Circulation of the medium was obtained by a peristaltic pump. The bioreactor was further equipped with an additional sampling point and containers for continuous medium exchange.

Production of 3D-adherent cells (PLX) - Non-confluent primary human adherent 2D cell cultures, grown as described above, were trypsinized, washed,

resuspended in DMEM supplemented with 10 % FBS, Pen-Strep-Nystatin mixture (100 U/ml:100 ug/ml:1.25 un/ml) and 2 mM L-glutamine, and seeded (10^3-10^5 cells/ml) via an injection point onto the 3D carriers in an aseptic Plug Flow bioreactor. Prior to inoculation, bioreactor was filled with PBS-Ca-Mg (Biological Industries, Beit Ha'emek, Israel), autoclaved (120 °C, 30 min) and washed with Dulbecco's growth medium containing 10 % heat-inactivated fetal calf serum and a Pen-Strep-Nystatin mixture (100 U/ml:100 ug/ml:1.25 un/ml). Flow was kept at a rate of 0.1-5 ml/min. Seeding process involved cease of circulation for 2- 48 hrs, thereby allowing the cells to settle on the carriers. Bioreactor was kept under controlled temperature (37 °C) and pH conditions (pH = 6.7-7.4); using an incubator supplied with sterile air and CO_2 as needed. Growth medium was replaced 2-3 times a week. Circulation medium was replaced with fresh DMEM media, every 4 hr to 7 days. At a density of 1 X 10^6-1 X 10^7 cells/ml (following 12-40 days of growth), total medium volume was removed from the bioreactor and bioreactor and carriers were washed 3-5 times with PBS. 3D-adherent cells were then detached from the carriers with Trypsin-EDTA; (Biological Industries, Beit Ha'emek, Israel; 3-15 minutes with gentle agitation, 1-5 times), and were thereafter resuspended in DMEM and cryopreserved.

EXAMPLE 2

Production of the placenta derived 2D adherent cells of the present invention

2D adherent cells were produced which exhibit different characteristics then the above described 3D adherent cells.

Materials and Experimental Methods

Manufacturing process of 2D adherent cells

Receipt of Human Tissue

All placentas obtained were received from the maternity ward under approval of the Helsinki Committee of the medical facility. Accordingly, all placenta donors signed an informed consent and Donor Screening and Donor Testing was performed (IPC1). Immediately after taking the placenta from the donor (during the caesarean procedure), it was placed in an aseptic plastic bag and then in a temperature-preserving box with ice packs

Recovery and Processing of adherent cells

To initiate the process, the placenta was cut into pieces under aseptic conditions under laminar flow hood, washed with Hank's buffer solution and incubated for 2-5 hours at 37 °C with 0.1 % Collagenase (1 mg Collagenase/ml tissue). 2D cell medium (2D-Medium comprising DMEM supplemented with 10 % FBS, fungizone 0.25 µg/ml and Gentamycine 50 µg/ml) was added and the digested tissue was roughly filtered through a sterile metal strainer, collected in a sterile beaker and centrifuged (10 minutes, 1200 RPM, 4 °C). Using gentle pipeting, suspended cells were then diluted with 2D-Medium supplemented with antibiotics, seeded in 175 cm^2 flasks and incubated at 37 °C in a tissue culture incubator under humidified condition supplemented with 5 % CO_2. Following 2-3 days, in which the cells were allowed to adhere to the flask surface, they were washed with PBS and 2D-Medium was added.

Two Dimensional (2D) Cell Growth

Prior to the first passage, growth medium samples of 10 % of the total flask number in quarantine was pooled and taken for mycoplasma testing (IPC2). If cells were found to be negative for Mycoplasma (EZ-PCR Mycoplasma kit, Biological Industries, Israel), cells were released from quarantine. After 1-2 additional passages, using 2D-Medium supplemented with antibiotics cells were transferred to the 2D production clean room (2DP). Once in Room 2DP, culture was continued for another 3-6 passages using 2D-Medium *without* antibiotics Throughout the process, cultures were grown in a tissue culture incubator under humidified conditions with 5 % CO2 at 37 °C. After a total of 6-9 passages (9-17 cell doublings), cells were collected and cryopreserved as the 2D-Cell Stock (2DCS).

The first passage was usually carried out after 7-15 days. Beginning at passage 2 and continuing until passage 6-8, cells were passaged when the culture reached 70-90 % confluence, usually after 4-5 days (1.5-2 doublings). The cells were detached from the flasks using 0.25 % trypsin-EDTA (4 minutes at 37 °C) and seeded in a culture density of $4 \pm 0.5 \times 10^3$ cells/cm^2. The size of the tissue culture flasks raised as the passages proceed. The culturing process started in 175 cm^2 tissue culture flask, continued in 500 cm^2 (Triple flask) and finally the cells were seeded into Cell Factory 10 tray (6320 cm^2).

Prior to cryopreservation, at the end of 2DCS growth period, the growth medium was collected and the sample was prepared to be sent to an approved GLP laboratory for Mycoplasma test (IPC 4).

Cryopreservation Procedure for 2D-Cell-Stock Product

For 2DCS cryopreservation, 2D-cultured cells were collected under aseptic conditions using 0.25 % trypsin-EDTA. The cells were centrifuged (1200 RPM, 10', 4 °C), counted and re-suspended in 2D-Medium.

For freezing, cell suspensions were diluted 1:1 with 2D-Freezing Mixture (final concentrations was 10 % DMSO, 40 % FBS and 50 % 2D-Medium). Approximately 1.5 - 2.5 x 10^9 cells were manufactured from one placenta. 4 ml of the cells were stored at a final concentration of 10 x 10^6/ ml in 5 ml cryopreservation polypropylene vials. The vials were labeled and transferred to a controlled rate freezer for a graduated temperature reducing process (1 °C/min), after which they were transferred to storage in gas-phase of a liquid nitrogen freezer. This material was referred to as the 2D-Cell Stock (2DCS) batch.

EXAMPLE 3

Comparison of the 3D adherent cells (PLX) to the 2D adherent cells of the present invention

3D adherent cells (PLX) produced as described hereinabove were compared to the new 2D adherent cells of the present invention.

Materials and Experimental Methods

Cell Cycle analysis – 2D adherent cells and PLX cells were fixed with 70 % EtOH O.N, centrifuged and re-suspended in a Propidium Iodide (PI) solution containing 2 µg/ml PI (Sigma), 0.2 mg/ml Rnase A (Sigma) and 0.1 % (v/v) Triton (Sigma) for 30 minutes.. Cell cycle was analyzed by FACS.

Gene expression array (Microarray) - Adherent cells were obtained from human full term placentas and were expanded by 2D cultures or according to the teachings of WO/2007/108003 (as described in detail in Examples 1-2). Three different batches of cells were obtained from each of the expansion methods for further examination.

RNA was extracted from the cells (Qiagen- Rneasy micro kit) and applied to an Affymetrix whole genome expression array GeneChip® Human Exon 1.0 ST Array (Affymetrix, Santa Clara, California, USA)..

FACS analysis of membrane markers - cells were stained with monoclonal antibodies as previously described. In short, 400,000-600,000 cells were suspended in 0.1 ml flow cytometer buffer in a 5 ml test tube and incubated for 15minutes at room temperature (RT), in the dark, with each of the following monoclonal antibodies (MAbs): FITC-conjugated anti-human CD29 MAb (eBioscience), PE conjugated anti human CD73 MAb (Becton Dickinson) ,PE conjugated anti human CD105 MAb (eBioscience), PE conjugated anti human CD90 MAb (Becton Dickinson), FITC-conjugated anti-human CD45 MAb (IQProducts), PE-conjugated anti-human CD19 MAb (IQProducts), PE conjugated anti human CD14 MAb (IQProducts), FITC conjugated anti human HLA-DR MAb (IQProduct), PE conjugated anti human CD34 MAb (IQProducts), FITC conjugated anti human CD31 MAb (eBioscience), FITC conjugated anti human KDR MAb (R&D systems), anti human fibroblasts marker (D7-FIB) MAb(ACRIS), FITC-conjugated anti-human CD80 MAb (BD), FITC-conjugated anti-human CD86 MAb (BD), PE conjugated anti-human CD200 MAb (BD), FITC-conjugated anti-human CD40 MAb (BD), FITC-conjugated anti-human HLA-ABC MAb (BD), Isotype IgG1 FITC conjugated (IQ Products), Isotype IgG1 PE conjugated (IQ Products).

Cells were washed twice with flow cytometer buffer, resuspended in 500 µl flow cytometer buffer and analyzed by flow cytometry using FC-500 Flow Cytometer (Beckman Coulter). Negative controls were prepared with relevant isotype fluorescence molecules.

Immunomodulation assay

Human derived mononuclear cells (MNCs) were isolated from peripheral blood. Suspension of 200,000 MNCs per 200 µl medium (RPMI 1640 medium containing 20 % FBS per 96 well) were stimulated with 10 µg PHA/ml (SIGMA) in the presence of 20,000 2D adherent cells for 5 days under humidified 5 % CO_2 at 37 °C. Four different batches of 2D adherent cells were used. Three replicates of each group were seeded in 96-well plated. During the last 18 hrs of the 5 - day culture, cells were pulsed with 1 µC

^{3}H-thymidine and further harvested over fiberglass filter. Thymidine uptake was quantified by a scintillation counter.

Experimental results

As is illustrated in Table 1, below, processing of the 2D adherent cells of the present invention differed from the 2D stage of PLX (WO/2007/108003) in a few aspects. First, the new 2D adherent cell's culture medium is supplemented with antibiotics only during the initial culturing stage (up to passage 2). Also, the new 2D adherent cells are cryopreserved only after 5-8 passages (i.e. at the end of culture) and not, as in the PLX process, during intermediate stages of 2D growth.

Table 1: Comparison of the 2D adherent cells of the present invention to those produced for PLX in WO/2007/108003

Parameter	WO/2007/108003	2D adherent cells of the present invention
Tissue culture flask	80 cm^2 and 175 cm^2	175 cm^2, triple flasks and Multi Tray
Medium supplemented with antibiotics	In all stages of the process	Up to passage 2 (included)
Cryopreservation of 2DCS	After 2-3 passages, then cryopreserved, thawed and seeded for a secondary growth in flasks for 2-5 passages, prior to seeding in bioreactor	After 5-8 passages, then cryopreserved and thawed prior to use
Freezing container	2 ml cryogenic vials	5 ml cryogenic vials
Freezing volume	1-1.5 ml	4 ml
Freezing method	Freezing container (contains isopropyl alcohol)	Controlled rate freezer

Changes in the manufacturing process of the new 2D adherent cells resulted in changes in characteristics of the obtained cells. These differences are summarized hereinbelow.

Cell cycle analysis of 2D adherent cells compared to 3D adherent cells of WO/2007/108003– 2D adherent cells were compared to 3D adherent cells in order to examine the distribution of the cells between the different phases of the cell cycle. As is clear from Figures 1A-B, 2D adherent cells exhibited typical proliferating profile

(distribution of cells between the different phases of cell cycle). Specifically, 28 % of cells were in S and G2/M phases (Figure 1A). These results indicated that cells were harvested during proliferation and that the culturing conditions supported cell growth.

Conversely, 3D adherent cells exhibited lower rates of proliferating cells. Less than 8 % of cells were in S and G2/M phases (Figure 1B). These results indicated that cells were harvested while low levels of proliferation were taking place and suggest that conditions in the bioreactor were suboptimal to support cell growth.

Microarray comparison between cells obtained by the teachings of WO/2007/108003 and by cells obtained by the teachings of the present invention - gene expression arrays enabled to simultaneously monitor genome-wide expression profiles of adherent cells derived from human full term placentas expanded by 2D cultures or according to the teachings of WO/2007/108003 (PLX, see Example 1, hereinabove). These results enabled to asses the molecular mechanism underlying phenotypic variation between cells obtained by these different growth methods (see Table 2, below).

Table 2: Gene expression in 2D adherent cells of the present invention compared to those expressed by PLX of WO/2007/108003

Gene	2D vs. Plurix (fold change)	p-value(treat)
interferon-induced protein with tetratricopeptide repeats	**21.82**	0.0401812
leukocyte-derived arginine aminopeptidase	**14.56**	3.88E-06
signal peptide, CUB domain, EGF-like 3	**10.82**	0.0255115
dickkopf homolog 1 (Xenopus laevis)	**6.84**	3.06E-07
integrin, alpha 6	**6.76**	0.0411667
keratin 27 pseudogene 27	**6.39**	0.000224998
similar to Keratin, type I cytoskeletal 18 (Cytokerati	**6.24**	0.000304949
aldehyde dehydrogenase 1 family, member A1	**5.84**	0.00145807
G protein-coupled receptor, family C, group 5, member A	**5.75**	3.39E-05
coagulation factor III (thromboplastin, tissue factor)	**5.55**	0.012192
cyclin-dependent kinase inhibitor 3 (CDK2-associated dual	**5.51**	0.000732492
G protein-coupled receptor 126	**5.50**	0.00197635
DEP domain containing 1	**5.41**	0.000370513
SHC SH2-domain binding protein 1	**4.96**	0.00430878
centrosomal protein 55kDa	**4.78**	0.0021952
interferon-induced protein with tetratricopeptide repeats	**4.66**	0.0139777
NUF2, NDC80 kinetochore complex component, homolog (S. cere	**4.61**	0.00276524
mal, T-cell differentiation protein-like	**4.44**	0.00664216

interferon-induced protein with tetratricopeptide repea	**4.42**	0.00357376
kinesin family member 18A	**4.33**	0.00134108
cholinergic receptor, muscarinic 2	**4.07**	0.0320078
cell division cycle 2, G1 to S and G2 to M	**4.06**	0.0017111
non-SMC condensin I complex, subunit G	**4.06**	0.00537097
denticleless homolog (Drosophila)	**4.06**	0.00141153
shugoshin-like 1 (S. pombe)	**4.00**	0.00101318
chromosome 13 open reading frame 3	**3.98**	0.000548296
PDZ binding kinase	**3.97**	0.00784983
lymphocyte cytosolic protein 1 (L-plastin)	**3.97**	0.0049584
WAS	**3.96**	0.00178153
cyclin E2	**3.94**	0.000203389
cathepsin C	**3.93**	0.00532262
integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4	**3.91**	0.0158411
KIAA0101	**3.90**	0.0105909
kinesin family member 20A	**3.90**	0.00582352
opioid growth factor receptor-like 1	**3.87**	0.00114551
anillin, actin binding protein	**3.83**	0.010923
catenin (cadherin-associated protein), alpha-like 1	**3.76**	7.46E-05
cell division cycle 20 homolog (S. cerevisiae)	**3.70**	0.00514206
diaphanous homolog 3 (Drosophila)	**3.69**	0.00107709
family with sequence similarity 111, member B	**3.69**	0.000125819
aurora kinase A	**3.66**	0.00632571
fibroblast growth factor 7 (keratinocyte growth factor)	**3.64**	0.0328983
maternal embryonic leucine zipper kinase	**3.63**	0.00908391
Rho GDP dissociation inhibitor (GDI) beta	**3.63**	0.00200066
centromere protein N	**3.62**	0.000540143
MAD2 mitotic arrest deficient-like 1 (yeast)	**3.62**	0.00488102
thymidylate synthetase	**3.61**	0.00685584
cyclin B2	**3.60**	0.016544
regulator of G-protein signalling 4	**3.59**	0.00781061
chromosome 6 open reading frame 173	**3.58**	0.00222408
hyaluronan-mediated motility receptor (RHAMM)	**3.55**	0.00467816
BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast	**3.54**	0.0108258
SPC25, NDC80 kinetochore complex component, homolog (S. ce	**3.53**	0.00568662
establishment of cohesion 1 homolog 2 (S. cerevisiae)	**3.52**	0.000773033
cyclin A2	**3.51**	0.00965934
CDC28 protein kinase regulatory subunit 2	**3.51**	0.0128024
keratin 18	**3.47**	0.000514523
ribonucleotide reductase M2 polypeptide	**3.46**	0.00834059
arylacetamide deacetylase-like 1	**3.44**	0.000902645
kinesin family member 11	**3.43**	0.00915145
Rho GTPase activating protein 11A	**3.41**	0.00834174
GINS complex subunit 1 (Psf1 homolog)	**3.39**	0.00104515
discs, large homolog 7 (Drosophila)	**3.38**	0.0317074
TTK protein kinase	**3.38**	0.0112171
deleted in lymphocytic leukemia, 2	**3.38**	0.0109528
replication factor C (activator 1) 3, 38kDa	**3.37**	0.00109668

solute carrier family 7, (cationic amino acid transporte	**3.36**	0.00688017
dual-specificity tyrosine-(Y)-phosphorylation regulated ki	**3.34**	0.0234606
kinesin family member 2C	**3.34**	0.0059888
heat shock 22kDa protein 8	**3.32**	0.0219583
polo-like kinase 1 (Drosophila)	**3.30**	0.0140309
v-myb myeloblastosis viral oncogene homolog (avian)-lik	**3.28**	0.0043878
trypsinogen C	**3.28**	0.00416276
thymidine kinase 1, soluble	**3.27**	0.00124134
NAD(P)H dehydrogenase, quinone 1	**3.27**	0.000282423
high-mobility group box 2	**3.24**	0.0196872
cell division cycle associated 2	**3.24**	0.0122226
apolipoprotein B mRNA editing enzyme, catalytic polypep	**3.23**	0.00308692
serpin peptidase inhibitor, clade B (ovalbumin), member	**3.22**	0.0190218
guanine nucleotide binding protein (G protein), gamma 11	**3.22**	0.00140559
chromosome 15 open reading frame 23	**3.21**	0.000147331
kinesin family member 14	**3.19**	0.00947901
transmembrane protein 154	**3.18**	0.0045589
glycerol kinase	**3.16**	2.66E-05
KIAA1524	**3.15**	0.0380688
coagulation factor XIII, B polypeptide	**3.14**	0.0294465
tight junction protein 2 (zona occludens 2)	**3.13**	0.00012562
nei endonuclease VIII-like 3 (E. coli)	**3.12**	0.00115606
pleckstrin 2	**3.11**	0.0304429
kinesin family member 23	**3.09**	0.00790585
Rac GTPase activating protein 1	**3.09**	0.00381613
keratinocyte growth factor-like protein 1	**3.07**	0.0300588
keratinocyte growth factor-like protein 1	**3.07**	0.0300588
keratinocyte growth factor-like protein 1	**3.07**	0.0300588
transcription factor 19 (SC1)	**3.07**	0.00109627
OCIA domain containing 2	**3.07**	0.00122147
lung cancer metastasis-associated protein	**3.06**	0.00148024
transcription factor 19 (SC1)	**3.05**	0.00124327
transcription factor 19 (SC1)	**3.05**	0.00124327
Rho GTPase activating protein 29	**3.05**	0.0466211
glucosaminyl (N-acetyl) transferase 1, core 2 (beta-1,6-N-	**3.05**	0.0197148
replication factor C (activator 1) 4, 37kDa	**3.04**	0.00164152
protein regulator of cytokinesis 1	**3.01**	0.0325664
transforming, acidic coiled-coil containing protein 3	**2.98**	0.0014577
cancer susceptibility candidate 5	**2.96**	0.0330594
nucleolar and spindle associated protein 1	**2.96**	0.00520875
cyclin B1	**2.96**	0.0103092
transmembrane protein 48	**2.96**	0.00458248
ZW10 interactor	**2.95**	1.88E-05
endonuclease domain containing 1	**2.95**	0.000429245
hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan synd	**2.94**	0.000634057
fucosidase, alpha-L- 2, plasma	**2.94**	0.00540929
ubiquitin-conjugating enzyme E2T (putative)	**2.93**	0.00741886
lipase A, lysosomal acid, cholesterol esterase (Wolman dise	**2.92**	0.0167385

villin 2 (ezrin)	**2.92**	0.0131934
glycerol kinase	**2.90**	3.37E-06
WD repeat domain 76	**2.89**	0.0023531
CD97 molecule	**2.89**	0.00994045
chromosome 18 open reading frame 24	**2.89**	0.00347442
topoisomerase (DNA) II alpha 170kDa	**2.89**	0.0321109
integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3	**2.87**	0.00574148
family with sequence similarity 29, member A	**2.85**	0.00111165
kinesin family member 4A	**2.85**	0.0114203
BRCA1 associated RING domain 1	**2.85**	0.000540414
serum	**2.84**	0.0387246
RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae)	**2.83**	0.000854739
Fanconi anemia, complementation group I	**2.83**	0.00464532
dihydrofolate reductase	**2.82**	0.00178879
claspin homolog (Xenopus laevis)	**2.81**	0.00683624
ornithine decarboxylase 1	**2.81**	0.00144868
sperm associated antigen 5	**2.80**	0.00906321
histone cluster 1, H3b	**2.80**	0.0304598
ATPase family, AAA domain containing 2	**2.79**	0.00415258
KIAA0286 protein	**2.79**	0.00130563
guanine nucleotide binding protein (G protein), alpha inhi	**2.76**	0.00184597
BUB1 budding uninhibited by benzimidazoles 1 homolog beta	**2.74**	0.0166047
dihydrofolate reductase pseudogene	**2.74**	0.00141306
brix domain containing 1	**2.73**	0.00471977
cytoskeleton associated protein 2	**2.72**	0.0030499
mitochondrial ribosomal protein S28	**2.72**	0.00298194
polymerase (DNA directed), epsilon 2 (p59 subunit)	**2.72**	0.00479612
family with sequence similarity 72, member A	**2.72**	0.00143248
EBNA1 binding protein 2	**2.70**	0.00296292
similar to 40S ribosomal protein SA (P40) (34	**2.70**	0.0385298
adipose differentiation-related protein	**2.70**	0.000331751
thioredoxin reductase 1	**2.70**	0.000197486
minichromosome maintenance complex component 5	**2.69**	0.00475504
von Hippel-Lindau binding protein 1	**2.69**	0.00329061
SCL	**2.68**	0.00390288
Fanconi anemia, complementation group D2	**2.68**	0.0281405
NIMA (never in mitosis gene a)-related kinase 2	**2.68**	0.00289469
PHD finger protein 19	**2.68**	0.000177604
microsomal glutathione S-transferase 1	**2.68**	0.041701
breast cancer 2, early onset	**2.68**	0.00586847
non-SMC condensin I complex, subunit H	**2.67**	0.0216752
chromosome 13 open reading frame 27	**2.67**	0.0234588
histone cluster 1, H2bg	**2.67**	0.000180822
non-SMC condensin II complex, subunit G2	**2.66**	0.0130322
centromere protein I	**2.64**	0.0106816
stomatin	**2.64**	0.00387095
glutathione S-transferase omega 1	**2.63**	0.000648379
protein tyrosine phosphatase-like A domain containing	**2.62**	0.0419644
calcyclin binding protein	**2.62**	0.00524566

KIT ligand	**2.61**	0.00641955
ubiquitin-conjugating enzyme E2L 3	**2.61**	0.00343347
serpin peptidase inhibitor, clade B (ovalbumin), member	**2.60**	0.0030439
ATPase, Ca++ transporting, plasma membrane 4	**2.60**	0.023011
TPX2, microtubule-associated, homolog (Xenopus laevis)	**2.60**	0.0253137
thyroid hormone receptor interactor 13	**2.59**	0.0118319
H2A histone family, member Z	**2.59**	0.0129697
CDC28 protein kinase regulatory subunit 1B	**2.57**	0.0107391
cell division cycle associated 3	**2.57**	0.006289
minichromosome maintenance complex component 8	**2.57**	0.000841489
E2F transcription factor 2	**2.55**	0.0496479
TIMELESS interacting protein	**2.55**	0.00771062
minichromosome maintenance complex component 4	**2.54**	0.00342054
polo-like kinase 4 (Drosophila)	**2.53**	0.00209633
kinesin family member C1	**2.53**	0.00821937
dihydrofolate reductase	**2.52**	0.00307793
glycerol-3-phosphate dehydrogenase 2 (mitochondrial)	**2.52**	0.00211969
TGF beta-inducible nuclear protein 1	**2.51**	0.0365579
integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor	**2.51**	0.0210165
MLF1 interacting protein	**2.51**	0.0177203
heat shock 70kDa protein 2	**2.50**	0.0215102
hairy and enhancer of split 1, (Drosophila)	**2.50**	0.000283509
ATP-binding cassette, sub-family C (CFTR	**2.49**	0.00382491
serglycin	**2.48**	0.0443487
sema domain, immunoglobulin domain (Ig), short basic doma	**2.47**	0.008548
ankyrin repeat domain 1 (cardiac muscle)	**2.47**	0.00911953
transporter 1, ATP-binding cassette, sub-family B (MDR	**2.47**	0.00859077
transporter 1, ATP-binding cassette, sub-family B (MDR	**2.47**	0.00859077
transporter 1, ATP-binding cassette, sub-family B (MDR	**2.47**	0.00859077
histone cluster 1, H1b	**2.46**	0.0470898
family with sequence similarity 72, member A	**2.46**	0.00165234
membrane bound O-acyltransferase domain containing 1	**2.46**	0.01185
epidermal growth factor receptor pathway substrate 8	**2.45**	0.0194949
ASF1 anti-silencing function 1 homolog B (S. cerevisiae)	**2.45**	0.00543408
dedicator of cytokinesis 11	**2.44**	0.00697577
family with sequence similarity 72, member A	**2.44**	0.00162905
actin related protein 2	**2.44**	0.000288443
CTP synthase	**2.43**	8.80E-05
M-phase phosphoprotein 1	**2.43**	0.0271814
CDC28 protein kinase regulatory subunit 1B	**2.43**	0.0145263
histone cluster 1, H2ai	**2.43**	0.0161621
high-mobility group nucleosomal binding domain 2	**2.42**	0.0030536
heat shock 70kDa protein 1A	**2.42**	0.00734287
heat shock 70kDa protein 1A	**2.42**	0.00674816
carnitine palmitoyltransferase 1A (liver)	**2.41**	0.00170894
neurofilament, medium polypeptide 150kDa	**2.41**	0.0190611
transmembrane protein 62	**2.41**	0.00761064
vaccinia related kinase 1	**2.40**	0.0233182
geminin, DNA replication inhibitor	**2.40**	0.00167629

phosphoglucomutase 2	**2.40**	0.00818204
lamin B1	**2.40**	0.0477748
keratin 18	**2.40**	0.000112551
deafness, autosomal dominant 5	**2.39**	0.00235481
proteasome (prosome, macropain) subunit, beta type, 9 (lar	**2.39**	0.0202595
proteasome (prosome, macropain) subunit, beta type, 9 (lar	**2.39**	0.0202595
proteasome (prosome, macropain) subunit, beta type, 9 (lar	**2.39**	0.0202595
chromosome 12 open reading frame 31	**2.39**	0.0173089
isocitrate dehydrogenase 3 (NAD+) alpha	**2.39**	0.00297129
forkhead box M1	**2.38**	0.0203154
transmembrane protein 106C	**2.38**	0.000214223
hypothetical protein LOC729012	**2.38**	0.000446087
PHD finger protein 1	**2.37**	0.010191
mitochondrial ribosomal protein L15	**2.37**	0.0306092
elastin microfibril interfacer 2	**2.37**	0.0192072
hypothetical protein DKFZp762E1312	**2.37**	0.00726778
retinoblastoma-like 1 (p107)	**2.36**	0.00319946
tissue factor pathway inhibitor (lipoprotein-associated	**2.36**	0.0356227
epithelial cell transforming sequence 2 oncogene	**2.36**	0.000571152
crystallin, zeta (quinone reductase)	**2.36**	0.0370884
hect domain and RLD 4	**2.36**	0.00679184
high-mobility group nucleosomal binding domain 2	**2.36**	0.00384071
cell division cycle 25 homolog A (S. pombe)	**2.36**	0.000341692
thymopoietin	**2.35**	0.0223176
interferon-induced protein with tetratricopeptide repeats	**2.34**	0.0177928
Bloom syndrome	**2.34**	0.0209259
dual specificity phosphatase 1	**2.34**	0.00211272
elongation factor, RNA polymerase II, 2	**2.34**	0.0130017
small nuclear ribonucleoprotein D1 polypeptide 16kDa	**2.34**	0.0334665
CDC45 cell division cycle 45-like (S. cerevisiae)	**2.33**	0.00735977
exonuclease 1	**2.33**	0.00739393
ribosomal protein L39-like	**2.33**	0.00429384
histone cluster 1, H2bh	**2.33**	0.0377748
ribonucleotide reductase M1 polypeptide	**2.33**	0.000170076
sulfiredoxin 1 homolog (S. cerevisiae)	**2.32**	5.14E-05
multiple coagulation factor deficiency 2	**2.31**	0.0116892
proteasome (prosome, macropain) subunit, alpha type, 3	**2.31**	0.0195874
ribonuclease H2, subunit A	**2.30**	0.00669936
minichromosome maintenance complex component 10	**2.29**	0.0037925
heat shock 70kDa protein 1B	**2.28**	0.0048959
heat shock 70kDa protein 1B	**2.28**	0.0054404
heat shock 70kDa protein 1B	**2.28**	0.0054404
ATPase, Na+	**2.28**	0.000381464
hypothetical protein LOC201725	**2.28**	0.000313319
cathepsin L1	**2.27**	0.0314419
cell division cycle associated 5	**2.27**	0.01021
RAB8B, member RAS oncogene family	**2.27**	0.00417066
SPC24, NDC80 kinetochore complex component,	**2.27**	0.00287227

homolog (S. ce		
gamma-glutamyl hydrolase (conjugase, folylpolygammaglutamyl	**2.26**	0.0195219
cell division cycle 25 homolog C (S. pombe)	**2.25**	0.0169914
mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)	**2.25**	0.00578953
metallothionein 1L (gene	**2.25**	0.00709646
RRS1 ribosome biogenesis regulator homolog (S. cerevisiae)	**2.24**	0.0120061
cell division cycle associated 8	**2.24**	0.00619878
shugoshin-like 2 (S. pombe)	**2.24**	0.000852557
mRNA turnover 4 homolog (S. cerevisiae)	**2.24**	0.00373104
ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,	**2.24**	0.00830766
v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	**2.23**	0.0364123
replication factor C (activator 1) 2, 40kDa	**2.23**	0.00768959
NIMA (never in mitosis gene a)-related kinase 7	**2.23**	0.00159114
basic leucine zipper and W2 domains 2	**2.23**	0.0190782
histone cluster 1, H2bf	**2.23**	0.0124279
eukaryotic translation initiation factor 1A, X-linked	**2.23**	0.00330183
transporter 1, ATP-binding cassette, sub-family B (MDR	**2.22**	0.0164234
transporter 1, ATP-binding cassette, sub-family B (MDR	**2.22**	0.0164234
transporter 1, ATP-binding cassette, sub-family B (MDR	**2.22**	0.0164234
polymerase (RNA) III (DNA directed) polypeptide G (32kD)	**2.22**	0.0298794
phosphatidylinositol-4-phosphate 5-kinase, type II, alph	**2.22**	0.00964099
proteasome (prosome, macropain) 26S subunit, ATPase, 6	**2.22**	0.024269
pituitary tumor-transforming 1	**2.21**	0.0485166
histone cluster 2, H3d	**2.21**	0.0102932
sulfide quinone reductase-like (yeast)	**2.21**	0.0473641
serglycin	**2.20**	0.00880325
ribosomal protein L22-like 1	**2.20**	0.00335381
membrane protein, palmitoylated 1, 55kDa	**2.20**	0.000396285
solute carrier family 24 (sodium	**2.20**	0.0328774
STAM binding protein-like 1	**2.20**	0.0181743
WD repeat and HMG-box DNA binding protein 1	**2.20**	0.0034833
CSE1 chromosome segregation 1-like (yeast)	**2.20**	0.0013662
origin recognition complex, subunit 6 like (yeast)	**2.20**	0.00182466
transcription factor A, mitochondrial	**2.19**	0.0110092
exosome component 8	**2.19**	0.00132017
mitochondrial ribosomal protein L1	**2.19**	0.0361058
sphingomyelin synthase 2	**2.19**	0.0020701
deoxycytidine kinase	**2.18**	0.00101444
family with sequence similarity 29, member A	**2.18**	0.00469407
chromosome 6 open reading frame 167	**2.18**	0.0011095
dual specificity phosphatase 11 (RNA	**2.18**	0.00426788
F-box protein 45	**2.18**	0.00510098
ras-related C3 botulinum toxin substrate 2 (rho family, sma	**2.17**	0.0292466
FK506 binding protein 5	**2.17**	0.0193805
breast cancer 1, early onset	**2.17**	0.0180553

nuclear factor I	**2.17**	0.0010313
thioredoxin	**2.17**	0.009636
SH2 domain containing 4A	**2.16**	0.0323646
TGF beta-inducible nuclear protein 1	**2.16**	0.00285964
PSMC3 interacting protein	**2.16**	0.00766442
chromosome 3 open reading frame 14	**2.15**	0.0377617
polycomb group ring finger 5	**2.15**	0.000294142
centrosomal protein 27kDa	**2.15**	0.00931602
family with sequence similarity 64, member A	**2.14**	0.0019785
acidic (leucine-rich) nuclear phosphoprotein 32 family, m	**2.14**	0.0300263
sterol O-acyltransferase (acyl-Coenzyme A: cholesterol acy	**2.14**	0.0193637
TATA box binding protein (TBP)-associated factor, RNA poly	**2.13**	0.00514451
origin recognition complex, subunit 5-like (yeast)	**2.13**	0.049697
Rac GTPase activating protein 1 pseudogene	**2.13**	0.000269488
LSM5 homolog, U6 small nuclear RNA associated (S. cerevisia	**2.13**	0.00264664
minichromosome maintenance complex component 7	**2.13**	0.0457691
met proto-oncogene (hepatocyte growth factor receptor)	**2.13**	0.0318147
tripartite motif-containing 25	**2.13**	0.0456344
chromosome 13 open reading frame 34	**2.13**	0.000702936
patatin-like phospholipase domain containing 4	**2.13**	0.0168306
minichromosome maintenance complex component 6	**2.12**	0.0161279
intraflagellar transport 80 homolog (Chlamydomonas)	**2.12**	0.0247286
peptidylprolyl isomerase F (cyclophilin F)	**2.12**	0.00093846
UTP15, U3 small nucleolar ribonucleoprotein, homolog (S. c	**2.12**	0.00482559
TAF9B RNA polymerase II, TATA box binding protein (TBP)-as	**2.12**	0.0170365
TAF9B RNA polymerase II, TATA box binding protein (TBP)-as	**2.12**	0.0170365
ecotropic viral integration site 2B	**2.12**	0.0171408
3'-phosphoadenosine 5'-phosphosulfate synthase 2	**2.12**	1.43E-05
proteasome (prosome, macropain) activator subunit 2 (PA28	**2.12**	0.00609885
ADAM metallopeptidase with thrombospondin type 1 motif,	**2.12**	0.0102751
flap structure-specific endonuclease 1	**2.12**	0.006882
S100 calcium binding protein A3	**2.12**	0.0324073
RAD18 homolog (S. cerevisiae)	**2.11**	0.0016685
minichromosome maintenance complex component 3	**2.11**	0.0018389
exosome component 3	**2.11**	0.0249115
cysteinyl-tRNA synthetase 2, mitochondrial (putative)	**2.11**	0.00564558
glutamate-cysteine ligase, modifier subunit	**2.11**	0.00378868
brix domain containing 1	**2.11**	0.00981178
kinesin family member 22	**2.11**	0.0192406
UTP11-like, U3 small nucleolar ribonucleoprotein, (yeast)	**2.10**	0.0132794
v-ral simian leukemia viral oncogene homolog B (ras related	**2.10**	0.012225
meiotic nuclear divisions 1 homolog (S. cerevisiae)	**2.10**	0.00164447

phenylalanyl-tRNA synthetase, beta subunit	**2.10**	0.000245973
similar to Ubiquitin-conjugating enzyme E2S (Ubiqui	**2.10**	0.000415822
coiled-coil domain containing 68	**2.10**	0.00227586
lamin B receptor	**2.10**	0.000151784
Niemann-Pick disease, type C1	**2.10**	0.0108117
hydroxysteroid dehydrogenase like 2	**2.09**	3.71E-05
RMI1, RecQ mediated genome instability 1, homolog (S. cerev	**2.09**	0.00294705
overexpressed in colon carcinoma-1	**2.09**	0.015322
hypothetical protein FLJ20425	**2.09**	0.0174225
primase, polypeptide 1, 49kDa	**2.09**	0.00801018
chromosome 20 open reading frame 121	**2.09**	0.0146323
microtubule associated serine	**2.08**	0.00536974
endothelial differentiation, sphingolipid G-protein-coupled	**2.08**	0.0132848
homeobox A9	**2.08**	0.00520942
centromere protein L	**2.08**	0.000880856
nucleolar complex associated 3 homolog (S. cerevisiae)	**2.07**	0.000373346
fibroblast growth factor 7 (keratinocyte growth factor)	**2.07**	0.0173208
cysteine rich transmembrane BMP regulator 1 (chordin-like)	**2.07**	0.0267286
nucleoporin 155kDa	**2.07**	0.00218453
FLJ20105 protein	**2.06**	0.0127979
CD44 molecule (Indian blood group)	**2.06**	0.000651436
polymerase (DNA directed), alpha 2 (70kD subunit)	**2.06**	0.0033903
v-myb myeloblastosis viral oncogene homolog (avian)-like 2	**2.06**	0.00989416
origin recognition complex, subunit 1-like (yeast)	**2.06**	0.00207753
hypothetical protein FLJ25416	**2.06**	0.000177531
kinesin family member 22	**2.06**	0.0242075
heat shock 60kDa protein 1 (chaperonin)	**2.06**	0.0327412
minichromosome maintenance complex component 2	**2.05**	0.0021347
fumarylacetoacetate hydrolase (fumarylacetoacetase)	**2.05**	3.88E-05
glycerol kinase 3 pseudogene	**2.05**	0.0103203
retinitis pigmentosa 2 (X-linked recessive)	**2.05**	0.0264185
U2AF homology motif (UHM) kinase 1	**2.05**	0.0255167
chaperonin containing TCP1, subunit 5 (epsilon)	**2.04**	0.00125909
ATPase, H+ transporting, lysosomal 34kDa, V1 subunit D	**2.04**	0.0317453
transcription termination factor, RNA polymerase II	**2.04**	0.000393489
succinate-CoA ligase, GDP-forming, beta subunit	**2.04**	0.0028167
cyclin-dependent kinase inhibitor 1B (p27, Kip1)	**2.04**	0.00183021
tyrosine 3-monooxygenase	**2.04**	0.00021508
cofactor required for Sp1 transcriptional activation, subu	**2.04**	0.00141809
glycosyltransferase 8 domain containing 3	**2.03**	0.022868
ribosomal RNA processing 15 homolog (S. cerevisiae)	**2.03**	0.0274884
glycogenin 1	**2.03**	0.0224317
hypothetical protein FLJ40869	**2.03**	0.00444509
proliferating cell nuclear antigen	**2.03**	0.0031727
sterile alpha motif domain containing 12	**2.03**	0.0232188
chromosome 16 open reading frame 59	**2.03**	0.00185191
cofilin 2 (muscle)	**2.03**	0.0459235
eukaryotic translation initiation factor 2, subunit 2 bet	**2.03**	0.0139947

chromatin assembly factor 1, subunit B (p60)	**2.03**	0.0119687
Zwilch, kinetochore associated, homolog (Drosophila)	**2.02**	0.000725107
ATP-binding cassette, sub-family E (OABP), member 1	**2.02**	0.00454751
LSM3 homolog, U6 small nuclear RNA associated (S. cerevisia	**2.02**	0.0199824
IQ motif containing GTPase activating protein 3	**2.02**	0.0495882
tubulin, alpha 1c	**2.02**	0.00862586
DBF4 homolog (S. cerevisiae)	**2.01**	0.0458795
amyloid beta precursor protein binding protein 1	**2.01**	0.000910538
suppressor of variegation 3-9 homolog 1 (Drosophila)	**2.01**	0.00224324
THO complex 7 homolog (Drosophila)	**2.01**	0.0047251
amyotrophic lateral sclerosis 2 (juvenile) chromosome re	**2.01**	0.0484466
nucleoporin 37kDa	**2.01**	0.00652747
nucleolar protein 11	**2.01**	0.000852662
ATP synthase, H+ transporting, mitochondrial F0 complex	**2.01**	0.00866627
histone cluster 1, H2ai	**2.01**	0.0129155
phytoceramidase, alkaline	**2.01**	0.0157729
primase, polypeptide 2A, 58kDa	**2.01**	0.00290097
similar to High mobility group protein B1 (High mobili	**2.00**	0.000363158
mastermind-like 3 (Drosophila)	**-2.00**	0.00386667
UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylga	**-2.01**	0.0268634
ring finger protein 122	**-2.01**	0.0236621
chromodomain helicase DNA binding protein 3	**-2.01**	6.39E-05
centaurin, gamma-like family, member 10 pseudogene	**-2.01**	8.70E-05
chromosome 7 open reading frame 10	**-2.01**	0.00738442
chromosome 6 open reading frame 111	**-2.01**	0.0104492
centaurin, gamma-like family, member 10 pseudogene	**-2.01**	0.000334818
Prader-Willi syndrome chromosome region 1	**-2.01**	0.0415526
KIAA1245	**-2.01**	0.0186309
peroxidasin homolog (Drosophila)	**-2.01**	0.00219049
melanoma antigen family D, 4	**-2.02**	0.0263076
melanoma antigen family D, 4	**-2.02**	0.0263076
glucosidase, alpha; acid (Pompe disease, glycogen storage di	**-2.02**	0.000418401
phospholipase A2 receptor 1, 180kDa	**-2.03**	0.00069343
glycosyltransferase 8 domain containing 2	**-2.03**	0.0173546
KIAA1546	**-2.03**	0.000255634
protocadherin beta 9	**-2.03**	0.0285124
TBC1 domain family, member 3B	**-2.03**	0.000414974
sushi, nidogen and EGF-like domains 1	**-2.03**	0.00161129
microtubule-actin crosslinking factor 1	**-2.04**	0.00216
region containing neuroblastoma breakpoint family,	**-2.04**	0.0213393
golgi autoantigen, golgin subfamily a-like pseudogene	**-2.04**	0.0182674
transducin-like enhancer of split 4 (E(sp1) homolog, Drosop	**-2.04**	0.0164153
solute carrier family 22 (organic cation transporter),	**-2.05**	0.0137275
neighbor of Punc E11	**-2.05**	0.0184739
insulin-like growth factor binding protein 5	**-2.05**	0.011614
KIAA1245	**-2.06**	0.0185376
vitamin D (1,25- dihydroxyvitamin D3) receptor	**-2.06**	0.000192208

B-cell CLL	-2.06	0.00343507
KIAA1305	-2.06	0.00813727
KIAA1245	-2.06	0.0185609
centaurin, gamma-like family, member 10 pseudogene	-2.07	3.08E-05
TBC1 domain family, member 3B	-2.07	0.00141297
similar to TBC1 domain family member 3 (Rab GTPase-	-2.08	0.00105098
mannosidase, alpha, class 2B, member 1	-2.08	0.000353303
cysteine-rich PAK1 inhibitor	-2.08	0.000125336
midline 1 (Opitz	-2.08	0.00130803
small nucleolar RNA, H	-2.09	0.017124
urocortin 2	-2.09	0.00172263
neuroblastoma breakpoint family, member 11	-2.09	0.0138065
collagen, type VI, alpha 3	-2.09	2.09E-06
neuroblastoma breakpoint family, member 11	-2.09	0.0148372
hypothetical protein LOC646870	-2.09	0.0117625
calsyntenin 3	-2.09	0.00300887
cortactin binding protein 2	-2.09	2.28E-05
synaptic vesicle glycoprotein 2A	-2.10	0.00704212
similar to Dynamin-1 (D100) (Dynamin, brain) (B-dyn	-2.10	0.0190733
similar to Dynamin-1 (D100) (Dynamin, brain) (B-dyn	-2.10	0.0190733
similar to TBC1 domain family member 3 (Rab GTPase-	-2.10	0.00108467
Notch homolog 2 (Drosophila) N-terminal like	-2.10	0.0193058
matrix-remodelling associated 5	-2.11	0.000317637
complement component 1, s subcomponent	-2.11	0.0395863
cysteine sulfinic acid decarboxylase	-2.11	0.00428211
hypothetical protein FLJ36144	-2.11	0.00958437
hypothetical protein FLJ36144	-2.11	0.00958437
dihydropyrimidinase-like 3	-2.12	0.0165203
procollagen C-endopeptidase enhancer	-2.12	0.0039236
golgi autoantigen, golgin subfamily a-like pseudogene	-2.12	0.00720508
TBC1 domain family, member 3B	-2.12	0.00122924
collagen, type VII, alpha 1 (epidermolysis bullosa, dystr	-2.13	0.00109233
versican	-2.14	0.023885
mannose receptor, C type 2	-2.14	0.00012142
golgi autoantigen, golgin subfamily a-like pseudogene	-2.14	0.00767095
dynamin 1	-2.15	0.00139674
TBC1 domain family, member 3B	-2.16	0.00130459
PHD finger protein 21A	-2.17	0.00980401
centaurin, gamma-like family, member 10 pseudogene	-2.17	0.000180846
slit homolog 3 (Drosophila)	-2.17	0.02844
neuroepithelial cell transforming gene 1	-2.18	0.0109689
cyclin L2	-2.18	0.00093459
similar to dJ402H5.2 (novel protein similar to wo	-2.18	0.00621503
phospholipase D family, member 3	-2.18	1.17E-05
collagen, type VIII, alpha 1	-2.19	0.00187242
cyclin L2	-2.19	0.00109621
protocadherin beta 14	-2.20	0.0103892
matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase,	-2.20	5.59E-05
lysyl oxidase-like 4	-2.21	0.0120148
golgi autoantigen, golgin subfamily a-like pseudogene	-2.21	0.00977719
WW domain containing transcription regulator 1	-2.21	0.0379899

PDZ domain containing RING finger 3	-2.21	0.00931014
chromosome 14 open reading frame 37	-2.21	0.0182453
brain and acute leukemia, cytoplasmic	-2.22	0.0476919
calcium channel, voltage-dependent, L type, alpha 1C sub	-2.22	0.0189661
jun oncogene	-2.23	7.21E-05
interleukin 19	-2.23	0.0310328
centaurin, gamma-like family, member 10 pseudogene	-2.23	0.000595086
centaurin, gamma-like family, member 10 pseudogene	-2.23	0.000595086
---	-2.24	0.00666187
golgi autoantigen, golgin subfamily b, macrogolgin (with	-2.24	0.0164005
chromosome 15 open reading frame 51	-2.24	0.0123547
similar to Dynamin-1 (D100) (Dynamin, brain) (B-dyn	-2.24	0.0123547
similar to Dynamin-1 (D100) (Dynamin, brain) (B-dyn	-2.24	0.0123547
AE binding protein 1	-2.25	0.000105628
golgi autoantigen, golgin subfamily a-like pseudogene	-2.26	0.00770626
transmembrane protein 16A	-2.27	0.0481085
hypothetical LOC399844	-2.27	0.000491694
oculomedin	-2.27	0.00778869
low density lipoprotein-related protein 1 (alpha-2-macroglo	-2.28	4.26E-05
fibronectin leucine rich transmembrane protein 2	-2.28	0.0135122
phospholipid transfer protein	-2.29	0.00999206
similar to Dynamin-1 (D100) (Dynamin, brain) (B-dyn	-2.29	0.0122573
SATB homeobox 2	-2.31	0.039781
similar to TBC1 domain family member 3 (Rab GTPase-	-2.32	0.000870285
tweety homolog 1 (Drosophila)	-2.32	0.00450824
CD24 molecule	-2.34	0.0340122
chimerin (chimaerin) 1	-2.35	0.0287031
AHA1, activator of heat shock 90kDa protein ATPase homolog	-2.37	0.00979472
bicaudal C homolog 1 (Drosophila)	-2.38	0.0347162
solute carrier family 6 (neurotransmitter transporter, ta	-2.38	0.00729635
milk fat globule-EGF factor 8 protein	-2.39	0.000987073
WNK lysine deficient protein kinase 1	-2.40	1.57E-05
small nucleolar RNA, H	-2.41	0.00843141
tweety homolog 3 (Drosophila)	-2.42	0.000165552
SH3 and PX domains 2B	-2.42	0.0244357
WD repeat and SOCS box-containing 1	-2.44	0.0387851
hypothetical protein PRO2012	-2.45	0.00756704
golgi autoantigen, golgin subfamily a-like pseudogene	-2.46	0.00320764
microfibrillar-associated protein 2	-2.47	0.0152901
collagen, type XII, alpha 1	-2.47	0.000204664
ST6 beta-galactosamide alpha-2,6-sialyltranferase 2	-2.47	0.0216987
thioredoxin interacting protein	-2.48	0.0135494
latent transforming growth factor beta binding protein 2	-2.49	4.08E-05
golgi autoantigen, golgin subfamily a-like pseudogene	-2.49	0.00603583
formin binding protein 1-like	-2.50	0.00290401
maternally expressed 3	-2.52	0.0112259
PTK7 protein tyrosine kinase 7	-2.54	0.000116114
ribonuclease P RNA component H1	-2.57	0.0156126
sushi-repeat-containing protein, X-linked 2	-2.58	0.0253856

sortilin-related VPS10 domain containing receptor 2	**-2.58**	0.00936311
similar to RIKEN cDNA 1110018M03	**-2.59**	0.00516476
pyridoxal-dependent decarboxylase domain containing 2	**-2.60**	0.00683647
Enah	**-2.61**	0.0077547
asporin	**-2.62**	0.000659873
small Cajal body-specific RNA 17	**-2.63**	0.0301336
nuclear pore complex interacting protein	**-2.67**	0.00988632
sushi, von Willebrand factor type A, EGF and pentraxin dom	**-2.69**	2.23E-05
protein tyrosine phosphatase, receptor type, U	**-2.69**	0.0270428
collagen, type V, alpha 1	**-2.70**	0.0166427
nuclear pore complex interacting protein	**-2.73**	0.0018339
transformer-2 alpha	**-2.74**	0.012256
dystrophin related protein 2	**-2.79**	0.0137557
golgi autoantigen, golgin subfamily a, 8A	**-2.80**	0.0111179
collagen, type VI, alpha 2	**-2.81**	0.0149554
transforming growth factor, beta 3	**-2.81**	0.0287865
trophinin	**-2.82**	0.00298044
hypothetical protein MGC24103	**-2.86**	0.0346673
supervillin	**-2.87**	0.0412717
ADAM metallopeptidase with thrombospondin type 1 motif,	**-2.90**	0.0113968
kinesin family member 26B	**-2.91**	0.00363199
nuclear pore complex interacting protein	**-2.91**	0.00160273
trichorhinophalangeal syndrome I	**-2.94**	0.00557712
nuclear pore complex interacting protein	**-2.96**	0.00111223
small nucleolar RNA, C	**-2.96**	0.00666866
homeobox A2	**-2.97**	0.0435423
distal-less homeobox 5	**-3.00**	0.000640157
dachsous 1 (Drosophila)	**-3.00**	0.00697244
small nucleolar RNA, C	**-3.06**	0.0274043
small nucleolar RNA, C	**-3.06**	0.0274043
nuclear pore complex interacting protein	**-3.09**	0.00583397
small nucleolar RNA, C	**-3.14**	0.0104491
small nucleolar RNA, C	**-3.14**	0.0104491
sushi-repeat-containing protein, X-linked	**-3.16**	0.00370941
zinc finger protein 521	**-3.17**	0.00668815
nuclear pore complex interacting protein	**-3.17**	0.00117582
chromosome 9 open reading frame 3	**-3.18**	0.00410177
golgi autoantigen, golgin subfamily a, 8B	**-3.18**	0.0121417
hemicentin 1	**-3.21**	0.0461603
small nucleolar RNA, C	**-3.24**	0.00765575
Kallmann syndrome 1 sequence	**-3.25**	0.000548703
tenascin C (hexabrachion)	**-3.26**	8.26E-05
nuclear pore complex interacting protein	**-3.29**	0.00282604
nuclear pore complex interacting protein	**-3.34**	0.00263888
homeobox B2	**-3.36**	0.00665994
similar to nuclear pore complex interacting protein	**-3.41**	0.0187322
nuclear pore complex interacting protein	**-3.46**	0.00354416
cholesterol 25-hydroxylase	**-3.51**	0.0445558
ring finger protein 144	**-3.52**	0.0135334

nuclear pore complex interacting protein	**-3.55**	0.00316496
calbindin 2, 29kDa (calretinin)	**-3.56**	0.0290743
nuclear pore complex interacting protein	**-3.58**	0.00032839
nuclear pore complex interacting protein	**-3.60**	0.000414309
nuclear pore complex interacting protein	**-3.62**	0.00283418
nuclear pore complex interacting protein	**-3.64**	0.000213956
nuclear pore complex interacting protein	**-3.66**	0.000377834
KIAA1641	**-3.69**	0.0191782
UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylga	**-3.72**	0.00964109
nuclear pore complex interacting protein	**-3.73**	0.000352007
leucine rich repeat containing 17	**-3.75**	0.0263961
chromosome 9 open reading frame 3	**-3.80**	0.0233723
nuclear pore complex interacting protein	**-3.82**	0.00368967
neurotrimin	**-3.87**	3.78E-06
protein tyrosine phosphatase, receptor type, N	**-4.02**	0.0294569
KIAA1641	**-4.02**	0.00659194
---	**-4.06**	0.00488845
KIAA1641	**-4.16**	0.0170531
integrin, alpha 11	**-4.16**	0.000390317
KIAA1641	**-4.27**	0.013175
odz, odd Oz	**-4.28**	0.00172671
transmembrane protein 119	**-4.34**	0.00801387
plexin domain containing 2	**-4.44**	0.031799
ras homolog gene family, member J	**-4.59**	0.00197982
homeobox B3	**-4.60**	0.0354368
similar to Protein KIAA0220	**-4.72**	0.0302619
raftlin family member 2	**-4.79**	0.0260454
WNT1 inducible signaling pathway protein 1	**-5.99**	0.000672342
clusterin	**-6.40**	0.0303973
serpin peptidase inhibitor, clade F (alpha-2 antiplasmi	**-6.47**	0.00362941
sulfatase 2	**-6.58**	5.88E-05
hephaestin	**-6.74**	0.0123141
junctional adhesion molecule 2	**-7.33**	0.0306758
fibronectin type III domain containing 1	**-7.46**	0.0334696
sarcoglycan, delta (35kDa dystrophin-associated glycoprotein	**-7.69**	0.000881984
cystatin SN	**-8.27**	0.0496433
microfibrillar-associated protein 4	**-8.67**	0.00155578
biglycan	**-8.70**	0.00161284
transmembrane, prostate androgen induced RNA	**-10.54**	0.000100935
carboxypeptidase E	**-12.48**	0.00738131

Characterization of membrane markers on 2D adherent cells of the present teachings - the surface antigens expressed by 2D adherent cells were examined using monoclonal antibodies. These cells were stable adhesive cells that were expanded *in vitro* without the loss of phenotype and without showing signs of karyotypic changes.

Flow cytometric analysis of 2D adherent cell's membrane markers showed a high incidence of cells expressing CD105, CD73, CD90 and CD29. Furthermore, a high incidence of cells was lacking the expression of CD45, CD34 and CD19, CD11b, CD14, CD200 and HLA-DR surface markers (Figure 2).

Immunomodulation by 2D adherent cells – The immunogenicity of the 2D adherent cells was investigated next. As shown in Figure 3, four different batches of 2D adherent cells were capable of reducing lymphocyte proliferation, following mitogenic stimuli with Phytohemagglutinin (PHA), as was measured by Thymidine incorporation.

EXAMPLE 4

Osteocyte differentiation of 2D adherent cells

2D adherent cells from bone marrow or placenta origin were grown under osteocyte differentiation stimulating conditions.

Materials and Experimental Methods

Osteogenesis

Osteogenesis was carried out according to Chemicon osteogenesis kit (cat no. scr028, Millipore, MA, USA)

Osteogenesis induction medium

Osteogenesis induction medium was freshly made prior to each medium exchange using the kit components (See Table 3, below).

Table 3: Osteogenesis medium components

Component	Stock concentration	Amount	Final con
DMEM low glucose (Invitrogen, Gibco)		8.7 ml	87 %
Serum (heat inactivated)		1 ml	10 %
dexamethasone	1 mM	1 µl	0.1 µM
Asorbic Acid-2-Phosphate solution	0.1 M	20 µl	0.2 mM
Glycerol-2-Phosphate Solution	1 M	100 µL	10 Mm
L-glutamine	X 100	100 µl	X 1
Pen & Strep	X 100	100 µl	X 1

To arrive at 1 mM dexamethasone solution, 900 µl ethanol was added to 100 µl dexamethasone 10 mM solution. Stock solution was stored with the rest of the kit's components at -20 °C. A 50 ml serum vial was heat inactivated, divided into 5 ml aliquots and kept at -20 °C until use.

Coating 24-well tissue culture plates

A coating mixture comprising 12 μg/ml vitronectin and 12 μg/ml collagen (both included in the kit) was prepared by diluting each with 1 x PBS.

The coating mixture was then added to the wells to cover the well surfaces (5 wells x 2 plates were prepared). Plates were incubated overnight at room temperature. The coating mixture was then removed and the wells were rinsed once with PBS. Plates were aspirated right before use.

Cell Growth

Placenta derived cells (plc11-3-1) or bone marrow derived cells (BM108) were plated (200,000 cells per well) in 1 ml growth medium comprising DMEM (Invitrogen, Gibco), 10 % FCS (Invitrogen, Gibco), 2 Mm L-glutamine (Sigma-Aldrich), 45 μg/ml Gentamicin-IKA (Teva Medical) and 0.25 μg/ml Fungizone (Invitrogen, Gibco). Placenta derived cells (4 wells x 2 plates) or bone marrow derived cells (1 well x 2 plates) were grown until 100 % confluent (typically overnight) before initiating osteogenic differentiation.

When cells reached 100 % confluence, growth medium was aspirated and replaced with 1 ml osteogenesis induction medium (differentiation day 1). Osteogenesis induction medium was replaced with fresh medium every 2-3 days for a total of 14-17 days.

As a control, one of the two plates (for each of the cell types) was not incubated with osteogenesis differentiation medium but rather with the growth medium (described hereinabove).

On day 17, osteocytes were fixed and stained with Alizarin Red Solution as depicted in detail below.

Staining Protocol

Osteocyte staining was performed by first carefully aspirating the medium from each well (carefully as to not aspirate the cells). Cells were then fixed by incubating in iced cold 70 % ethanol for 1 hour at room temperature. The alcohol was then carefully aspirated and the cells were rinsed twice with water (5-10 minutes each wash). The water was then aspirated and alizarin red solution (500-1000 μl) was added to the cells. Cells were incubated with alizarin red solution at room temperature for 30 minutes. Alizarin red was removed and the cells were washed 4 times with 1 ml water and

aspirated after each wash. Finally, 1-1.5 ml water was added to each well to prevent cell drying. The plates were microscopically visualized by an inverted Nikon microscope.

Experimental results

Osteocyte differentiation of placenta- or bone marrow- derived adherent cells in osteogenic induction medium resulted in differentiation of over 50 % of the bone marrow cells, as demonstrated by positive alizarin red staining (Figure 4B). On the contrary, none of the placental derived cells showed any signs of osteogenic differentiation (see Figures 4B and 4E and Table 4, below).

Table 4: Differentiation summary

	BM 108+ BM109	PLC-11-3-1	PLC-8-2-1	Plc-15-3-4-2	Plc 4-3-1
Osteocytes	+++	-	-	-	-
Adipocytes	+++	-	-	-	-

EXAMPLE 5

Osteocyte differentiation of 2D adherent cells in modified growth medium

2D adherent cells from bone marrow or placenta origin were stimulated to differentiate in a modified osteogenic medium comprising Vitamin D and higher concentrations of dexamethasone.

Materials and Experimental Methods

Osteogenesis induction medium

Osteogenesis induction medium was freshly made prior to each medium exchange using the components listed in Table 5, below, along with Vitamin D.

Table 5: Osteogenesis medium components

Component	Stock con	Amount	Final con
DMEM high glucose (Biological Industries, Bet Haemek, Israel)		8.7 ml	87 %
L-glutamine	X 100	100 µl	X 1
Serum (heat inactivated)		1 ml	10 %
Dexamethasone (Chemicon)	10 mM	10 µl	10 µM
Asorbic Acid-2-Phosphate solution (Chemicon)	0.1 M	20 µl	0.2 mM

Glycerol-2-Phosphate Solution (Chemicon)	1 M	100 µL	10 Mm
Vitamin D (Sigma)	10 µM	10 µL	10 nM
Gentamycin (Biological Industries, Bet Haemek, Israel)	X 100	100 µl	X 1

A 50 ml serum vial was heat inactivated, divided into 5 ml aliquots and kept at -20 °C until use.

Coating 48-well tissue culture plates

A coating mixture comprising 12 µg/ml vitronectin and 12 µg/ml collagen (both from Chemicon) was prepared by diluting each with 1 x PBS.

The coating mixture was then added to the wells to cover the well surfaces (5 wells x 2 plates were prepared). Plates were incubated overnight at room temperature. The coating mixture was then removed and the wells were rinsed once with PBS. Plates were aspirated right before use.

Cell Growth

Placenta derived cells (PLC 8-2-1, PLC 15 3-4-2 or PLC 19-4-3-1 fetal cells) were plated (100,000 cells per well) in 0.5 ml growth medium comprising DMEM (Invitrogen, Gibco), 10 % FCS (Invitrogen, Gibco), 2 Mm L-glutamine (Sigma-Aldrich), 45 µg/ml Gentamicin-IKA (Teva Medical) and 0.25µg/ml Fungizone (Invitrogen, Gibco) (4 wells x 2 plates). Bone marrow derived cells (BM109) were plated (150,000 cells per well) in 0.5 ml growth medium (as described above) (1 well x 2 plates). Cells were grown until 100 % confluent (typically overnight) before initiating osteogenic differentiation.

When cells reached 100 % confluence, growth medium was aspirated and replaced with 0.5 ml osteogenesis induction medium (differentiation day 1). Osteogenesis induction medium was replaced with fresh medium every 2-3 days for a total of 26 days.

As a control, one of the two plates (for each of the cell types) was not incubated with osteogenesis differentiation medium but rather with the growth medium (described hereinabove).

On day 26, osteocytes were fixed and stained with Alizarin Red Solution as depicted in detail below.

Staining Protocol

Osteocyte staining was performed by first carefully aspirating the medium from each well (carefully as to not aspirate the cells). Cells were then fixed by incubating in iced cold 70 % ethanol for 1 hour at room temperature. The alcohol was then carefully aspirated and the cells were rinsed twice with water (5-10 minutes each wash). The water was then aspirated and alizarin red solution (500-1000 µl) was added to the cells. Cells were incubated with alizarin red solution at room temperature for 30 minutes. Alizarin red was removed and the cells were washed 4 times with 1 ml water and aspirated after each wash. Finally, 1-1.5 ml water was added to each well to prevent cell drying. The plates were microscopically visualized by an inverted Nikon microscope.

Experimental results

Osteogenic differentiation of placenta- or bone marrow- derived adherent cells was performed by modification of the protocol described in Example 4, hereinabove, according to previous teachings [Parloni et al. (2008) Stem Cells 26(2): 300-11]. The main difference between the growth conditions presented in Example 4 and the results presented herein was the addition of vitamin D to the differentiation medium and the higher concentrations of dexamethasone. As evident from the results, over 50 % of the bone marrow cells underwent differentiation into osteocytes, as demonstrated by positive alizarin red staining (see Figure 5B). However, none of the placental derived cells showed any signs of osteogenic differentiation (see Figure 5E and Table 4, hereinabove).

EXAMPLE 6

Adipocyte differentiation of 2D adherent cells

2D adherent cells from bone marrow or placenta origin were stimulated to differentiate into adipocytes.

Materials and Experimental Methods

Adipogenesis

Adipogenesis was carried out according to Chemicon adipogenesis kit (Chemicon adipogenesis kit, cat no. scr020, Millipore, MA, USA)

Adipogenesis induction medium

Adipogenesis induction or maintenance mediums were freshly prepared prior to every medium exchange using the components depicted in Tables 6 and 7, below.

Table 6: Adipogenesis induction medium components

Component	Stock con	Amount	Final con
DMEM low glucose (Biological Industries, Bet Haemek, Israel)		4.4 ml	90 %
Serum (heat inactivated)		0.5 ml	10 %
Dexamethasone (Sigma)	10 mM	0.5 µl	1 µM
IBMX (Sigma)	0.5 M	5 µl	0.5 mM
Insulin (Sigma)	10 mg/ml	5 µL	10 µg/ml
Indomethacin (Sigma)	10 mM	50 µl	100 µM
Pen & Strep	X 100	50 µl	X 1

Table 7: Adipogenesis maintenance medium components

Component	Stock con	Amount	Final con
DMEM low glucose		4.4 ml	90 %
Serum (heat inactivated)		0.5 ml	10 %
Insulin	10 mg/ml	5 µL	10 µg/ml
Pen & Strep	X 100	50 µl	X 1

Cell Growth

Placenta derived cells (plc11-3-1) or bone marrow derived cells (BM108) were plated (200,000 cells per well) in 1 ml growth medium comprising DMEM (Invitrogen, Gibco), 10 % FCS (Invitrogen, Gibco), 2 Mm L-glutamine (Sigma-Aldrich), 45 µg/ml Gentamicin-IKA (Teva Medical) and 0.25 µg/ml Fungizone (Invitrogen, Gibco). Placenta derived cells (4 wells x 2 plates) or bone marrow derived cells (1 well x 2 plates) were grown until 100 % confluent (typically overnight) before initiating adipogenesis differentiation.

When cells reached 100 % confluence, growth medium was aspirated and replaced with 1 ml adipogenesis induction medium (differentiation day 1). Adipogenesis induction medium was replaced with fresh medium every 2-3 days for a total of 25 days (as depicted in detail in Table 8, hereinbelow). Of note, monolayers of adipogenic cells were extremely fragile and could easily dislodged from plates, therefore, medium changes were performed with gentle medium changes to avoid disruption of the lipid droplets.

As a control, one of the two plates (for each of the cell types) was not incubated with adipogenesis differentiation medium but rather with the growth medium (described hereinabove).

Table 8: Adipogenesis differentiation schedule

Day	Medium
1	Adipogenesis *Induction* medium
3	Adipogenesis *Induction* medium
5	Adipogenesis *Induction* medium
7	Adipogenesis *Maintenance* medium
9	Adipogenesis *Induction* medium
11	Adipogenesis *Induction* medium
13	Adipogenesis *Induction* medium
15	Adipogenesis *Maintenance* medium
17	Adipogenesis *Induction* medium
19	Adipogenesis *Induction* medium
21	Adipogenesis *Induction* medium

On day 25, adipocytes were fixed and stained with oil red solution as depicted in detail below.

Staining Protocol

Adipocyte staining was performed by first carefully aspirating the medium from each well (carefully as to not aspirate the cells). Cells were then fixed by incubating in 4 % Para formaldehyde for 30-40 minutes at room temperature. The fixative was then carefully aspirated and the cells were rinsed three times with PBS (5-10 minutes each wash). Next, the PBS was aspirated and the cells were rinsed twice in water. The water was then aspirated and oil red solution (500-1000 µl) was added to the cells. Cells were incubated with oil red solution at room temperature for 50 minutes. Oil red solution was removed and the cells were washed 4 times with 1 ml water and aspirated after each wash. Finally, 1-1.5 ml water was added to each well to prevent cell drying. The plates were microscopically visualized by an inverted Nikon microscope.

Preparation of oil red solution

Stock of 0.25 g oil red (Sigma) was used which was dissolved in 50 ml iso-propanol by incubating10-15 min in 37 °C bath.

For use, 30 ml of the stock stain was mixed with 20 ml DDW (left to stand for 10 minutes and then filtered with coffee filter paper). The oil red solution was prepared fresh for each use.

Experimental results

Adipocyte differentiation of placenta- or bone marrow- derived adherent cells in adipocyte induction medium resulted in differentiation of over 50 % of the bone marrow derived cells (see Figure 4C), as demonstrated by positive oil red staining and by typical morphological changes (e.g. accumulation of oil droplets in the cytoplasm). In contrast, none of the placental derived cells differentiated into adipocytes (see Figure 4F and Table 4, hereinabove).

EXAMPLE 7

Adipocyte differentiation of 2D adherent cells in modified growth medium

2D adherent cells from bone marrow or placenta origin were stimulated to differentiate into adipocytes in a modified medium comprising a higher level of Indomethacine.

Materials and Experimental Methods

Adipogenesis induction medium

Adipogenesis induction medium was freshly prepared prior to every medium exchange using the components depicted in Table 9, below.

Table 9: Adipogenesis induction medium components

Component	Stock con	Amount	Final con
DMEM low glucose		4.4 ml	90 %
Serum (heat inactivated)		0.5 ml	10 %
Dexamethasone (Sigma)	1 mM	5 µl	1 µM
IBMX (Sigma)	0.5 M	5 µl	0.5 mM
Insulin (Sigma)	10 mg/ml	5 µL	10 µg/ml
Indomethacin (Sigma)	10 mM	200 µl	100 µM
Gentamycine (Biological Industries)		10 µl	

Cell Growth

Placenta derived cells (PLC 8-2-1, PLC 15 3-4-2 or PLC 19-4-3-1 fetal cells) were plated (100,000 cells per well) in 0.5 ml growth medium comprising DMEM

(Invitrogen, Gibco), 10 % FCS (Invitrogen, Gibco), 2 Mm L-glutamine (Sigma-Aldrich), 45 μg/ml Gentamicin-IKA (Teva Medical) and 0.25 μg/ml Fungizone (Invitrogen, Gibco) (5 wells x 2 plates).

Bone marrow derived cells (BM109) were plated (100,000 cells per well) in 0.5 ml growth medium comprising DMEM (Invitrogen, Gibco), 10 % FCS (Invitrogen, Gibco), 2 Mm L-glutamine (Sigma-Aldrich), 45 μg/ml Gentamicin-IKA (Teva Medical) and 0.25 μg/ml Fungizone (Invitrogen, Gibco) (4 well x 2 plates). Cells were grown until 100 % confluent (typically overnight) before initiating adipogenesis differentiation.

When cells reached 100 % confluence, growth medium was aspirated and replaced with 0.5 ml adipogenesis induction medium (differentiation day 1). Adipogenesis induction medium was replaced with fresh medium every 2-3 days for a total of 3-4 weeks.

As a control, one of the two plates (for each of the cell types) was not incubated with adipogenesis differentiation medium but rather with the growth medium (described hereinabove).

On day 26, adipocytes were fixed and stained with oil red solution as depicted in detail below.

Staining Protocol

Adipocyte staining was performed by first carefully aspirating the medium from each well (carefully as to not aspirate the cells). Cells were then fixed by incubating in 4 % Para formaldehyde for 30-40 minutes at room temperature. The fixative was then carefully aspirated and the cells were rinsed three times with PBS (5-10 minutes each wash). Next, the PBS was aspirated and the cells were rinsed twice in water. The water was then aspirated and oil red solution (500-1000 μl) was added to the cells. Cells were incubated with oil red solution at room temperature for 50 minutes. Oil red solution was removed and the cells were washed 3 times with 1 ml double distilled water and aspirated after each wash. Finally, 1-1.5 ml water was added to each well to prevent cell drying. The plates were microscopically visualized by an inverted Nikon microscope.

Preparation of oil red solution

Stock of 0.25 g oil red (Sigma) was used which was dissolved in 50 ml iso-propanol by incubating 10-15 min in 37 °C bath.

For use, 30 ml of the stock stain was mixed with 20 ml DDW (left to stand for 10 minutes and then filtered with coffee filter paper). The oil red solution was prepared fresh for each use.

Experimental results

Adipocyte differentiation of placenta- or bone marrow- derived adherent cells was performed by modification of the protocol in Example 6, hereinabove, according to previous teachings [Parloni et al. (2007), supra]. The main difference between the growth conditions presented in Example 6 and the results presented herein was the higher concentration of Indomethacine. As evident from the results, over 50 % of the bone marrow derived cells underwent differentiation into adipocytes (see Figure 5C), as demonstrated by positive oil red staining and by typical morphological changes (e.g. accumulation of oil droplets in the cytoplasm). In contrast, none of the placental derived cells exhibited morphological changes typical of adipocytes (see Figure 5F and Table 4, hereinabove).

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

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

WHAT IS CLAIMED IS:

1. A method of culturing adherent cells from a placenta or adipose tissue, the method comprising culturing the adherent cells from the placenta or adipose tissue under 2 dimensional (2D) culturing conditions which allow cell expansion, said conditions comprising continuous passaging of said cells for at least 4 passages.

2. The method of claim 1, further comprising growing the cells in a culture medium devoid of an antibiotic from at least passage 2.

3. A population of cells generated according to claims 1 or 2.

4. The population of cells of claim 3, wherein at least 12 % of said adherent cells are at a proliferative phase.

5. The population of cells of claim 3, wherein said adherent cells are capable of suppressing an immune reaction.

6. The population of cells of claim 3, wherein said adherent cells comprise a positive marker expression selected from the group consisting of CD73, CD90, CD29 and CD105.

7. The population of cells of claim 3, wherein said adherent cells comprise a negative marker expression selected from the group consisting of CD11b, CD34, HLA-DR, CD14, CD19, CD200 and CD45.

8. The population of cells of claim 3, wherein said adherent cells comprise a gene expression profile essentially as described herein.

9. The population of cells of claim 3, wherein said adherent cells are less committed to an osteogenic lineage as compared to adherent cells from bone marrow grown and allowed to differentiate under the same conditions.

10. The population of cells of claim 3, wherein said adherent cells are less committed to an adipogenic lineage as compared to adherent cells from bone marrow grown and allowed to differentiate under the same conditions.

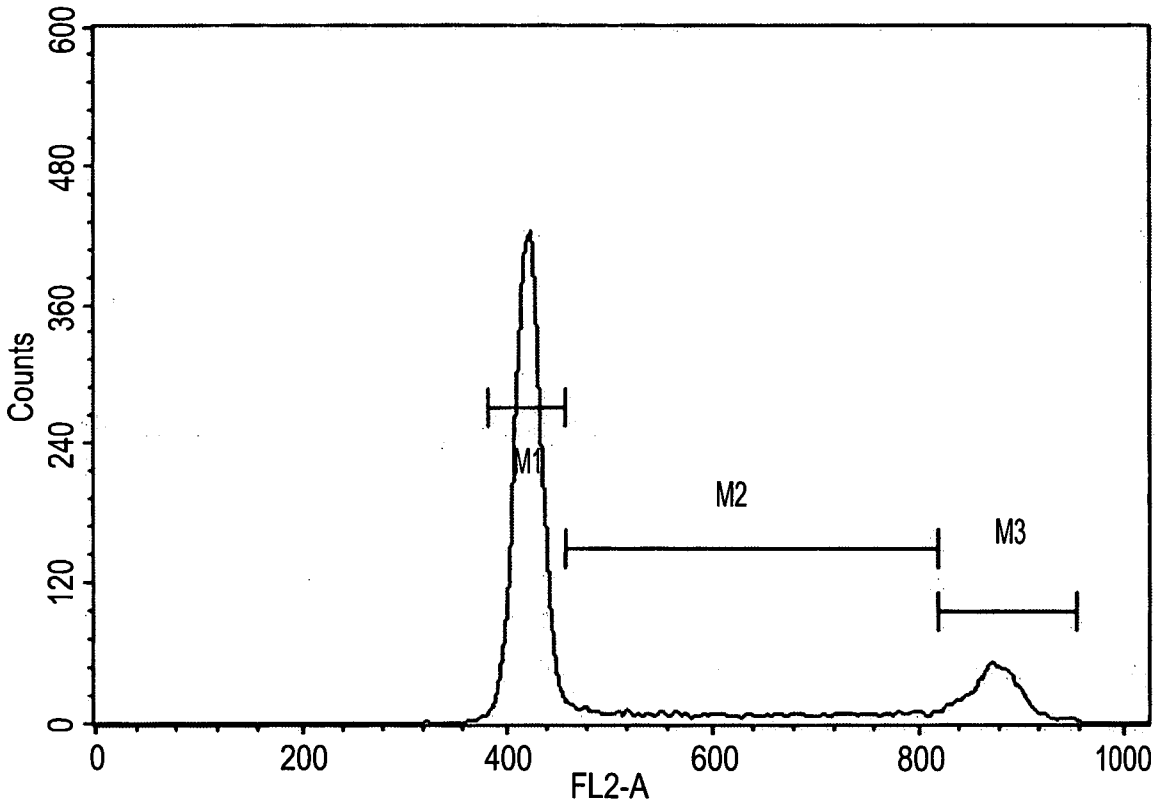
11. A population of cells comprising a gene expression profile essentially as described herein.

12. Use of the population of cells of any one of claims 1-11, for the manufacture of a medicament identified for treating a condition which can benefit from cell or organ transplantation.

13. The use of claim 12, wherein the condition is selected from the group consisting of ischemia, peripheral arterial disease (PAD), critical limb ischemia (CLI), lower extremity ischemia, ischemic vascular disease, vascular disease of the kidney, ischemic heart disease, myocardial ischemia, coronary artery disease (CAD), atherosclerotic cardiovascular disease, left main coronary artery disease, arterial occlusive disease, peripheral ischemia, peripheral vascular disease, arteriosclerosis, ischemic brain disease, stroke, cerebral ischemia, cerebro vascular disease, retinopathy, retinal repair, remodeling disorder, von Hippel-Lindau syndrome, hereditary hemorrhagic telengiectasiaischemic vascular disease, Buerger's disease, ischemic renal disease, ischemic placenta, reproduction associated disorders, graft-versus-host disease, solid organ transplantation, hematopoietic stem cell transplantation, diabetes, connective tissue damage, cancer, pre-cancer, bone cancer, osteosarcoma, bone metastases, bone fracture, burn wound, articular cartilage defect, deep wound, delayed wound-healing, delayed ulcer healing, subchondral-bone cyst, osteoporosis, osteoarthritis, degenerated bone, cartilage damage, articular cartilage defect, injured tendons, autoimmune diseases, metabolic disorders, psoriasis, neuropathic pain, peripheral nerve injury, support of kidney transplantation and inflammatory diseases.

14. A method of inducing tolerance and/or immunosuppression in a subject in need thereof, the method comprising administering to the subject a therapeutically

effective amount of adherent cells of claims 1-11, thereby inducing tolerance and/or immunosuppression in the subject.



Histogram Statistics

Log Data Units: Linear Values

Sample ID:
Patient Name:

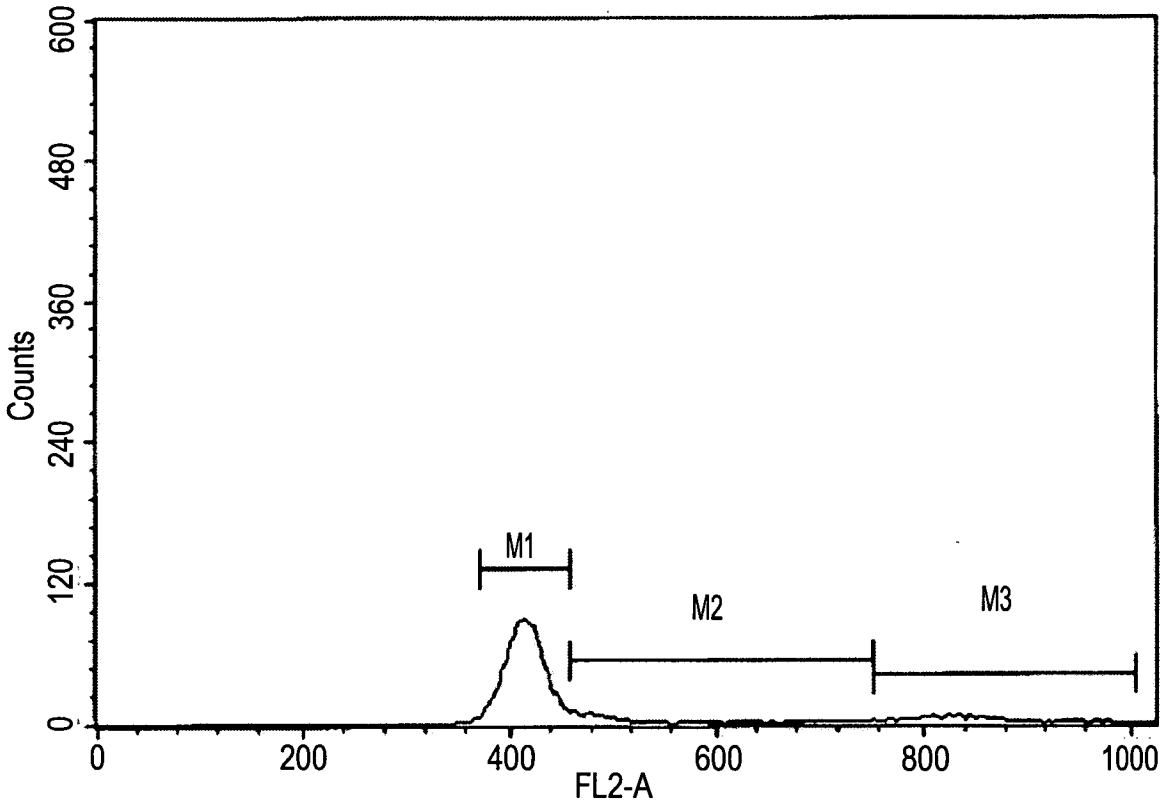
Patient ID:
Case Number:
Gate: G1

Gated Events: 18271
X Parameter: FL2-A (Linear)

Total Events: 20000

Marker	Left,	Right	Events	%Gated	%Total	Mean	Geo Mean	CV	Median	Peak Ch
All	0,	1023	18271	100.00	91.36	520.73	497.53	33.37	427.00	423
M1	382,	457	12698	69.50	63.49	420.10	419.91	2.99	420.00	423
M2	457,	818	2637	14.43	13.18	627.08	616.92	17.91	624.00	462
M3	818,	954	2879	15.76	14.39	871.73	871.36	2.92	871.00	867

FIG. 1A



Histogram Statistics

Log Data Units: Linear Values

Sample ID:
Patient ID:
Patient Name:
Case Number:
Gate: G1

Gated Events: 5202
Total Events: 15420
X Parameter: FL2-A (Linear)

Marker	Left,	Right	Events	%Gated	%Total	Mean	Geo Mean	CV	Median	Peak Ch
All	0,	1023	5202	100.00	33.74	474.23	458.74	29.62	419.00	413
M1	370,	457	3944	76.82	25.58	413.33	412.96	4.24	413.00	413
M2	457,	752	597	11.48	3.87	538.87	531.96	16.91	494.00	464
M3	752,	1005	591	11.36	3.83	833.73	832.39	5.75	827.00	829

FIG. 1B

FIG. 2

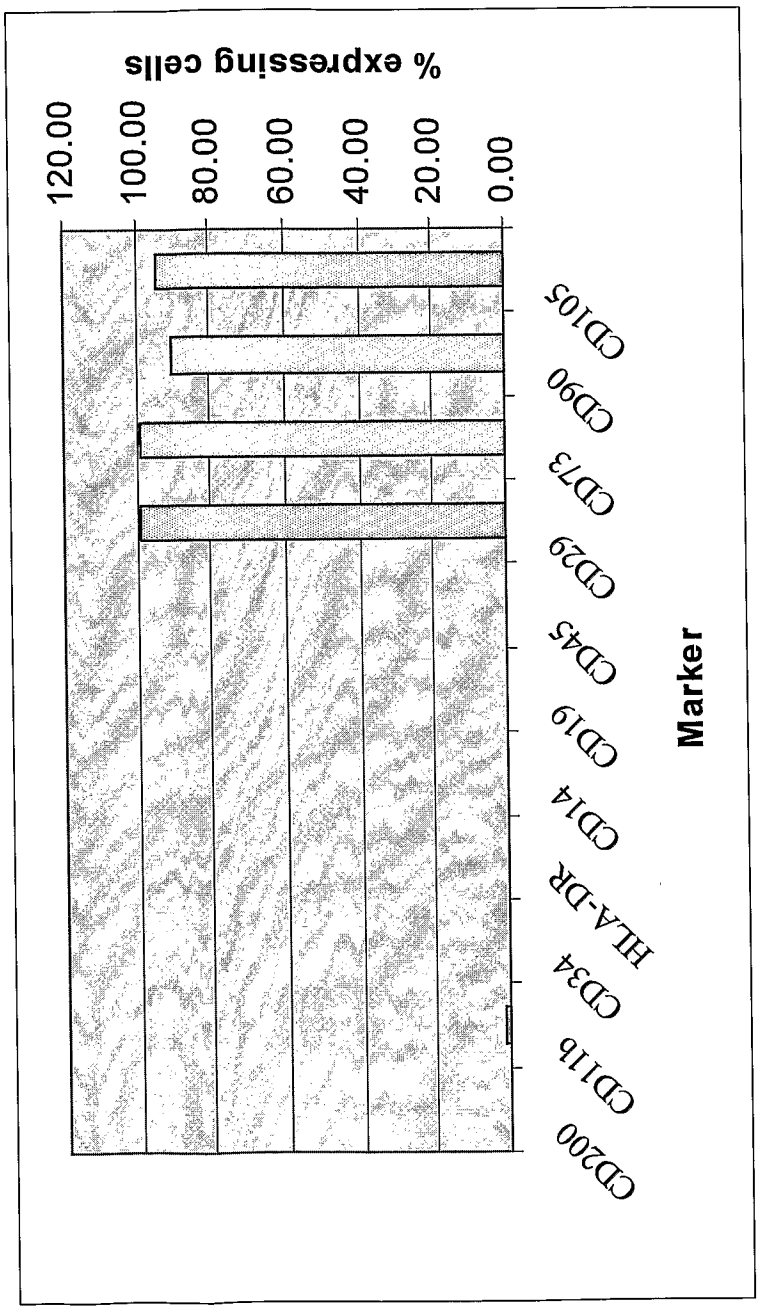
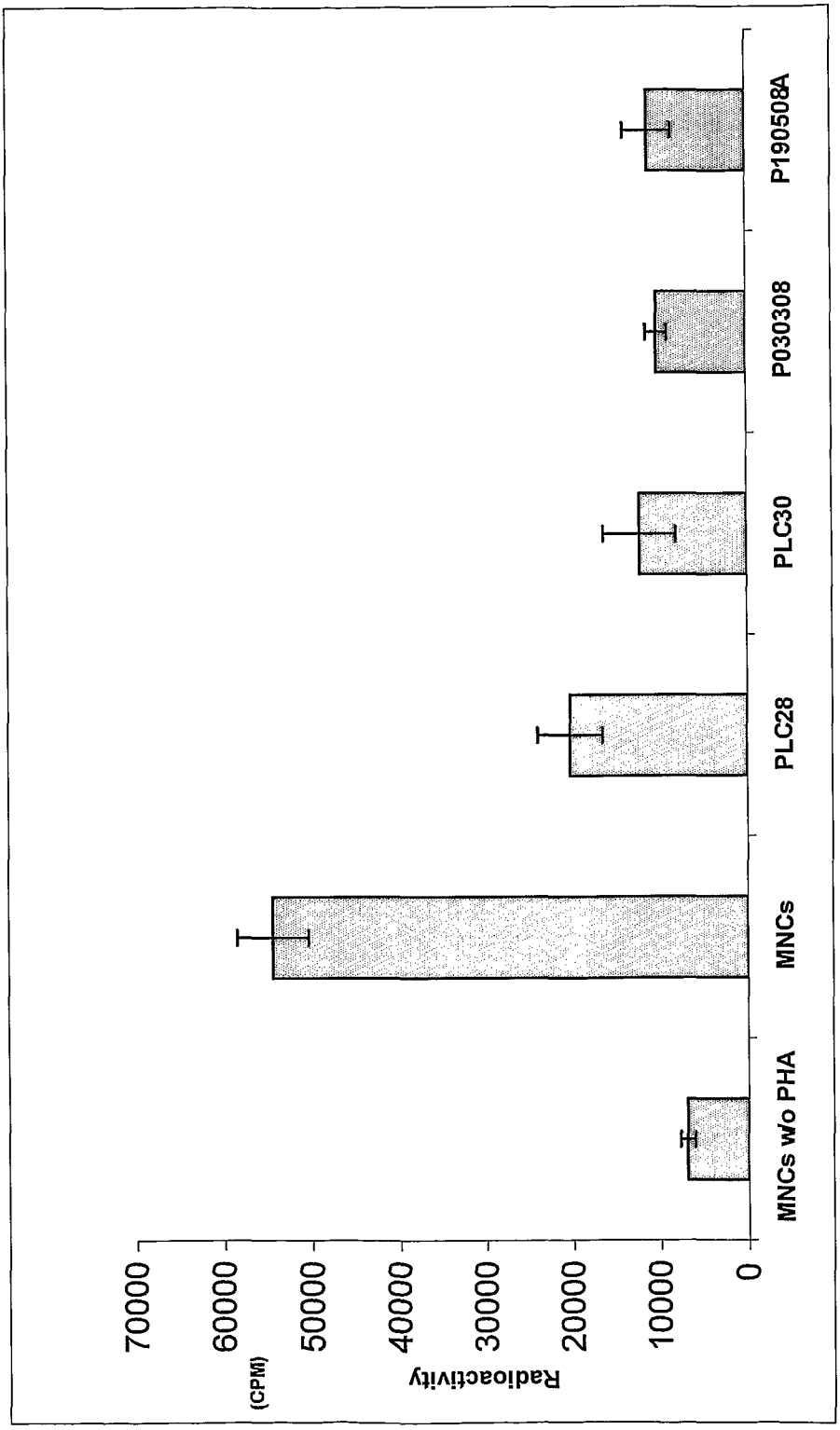
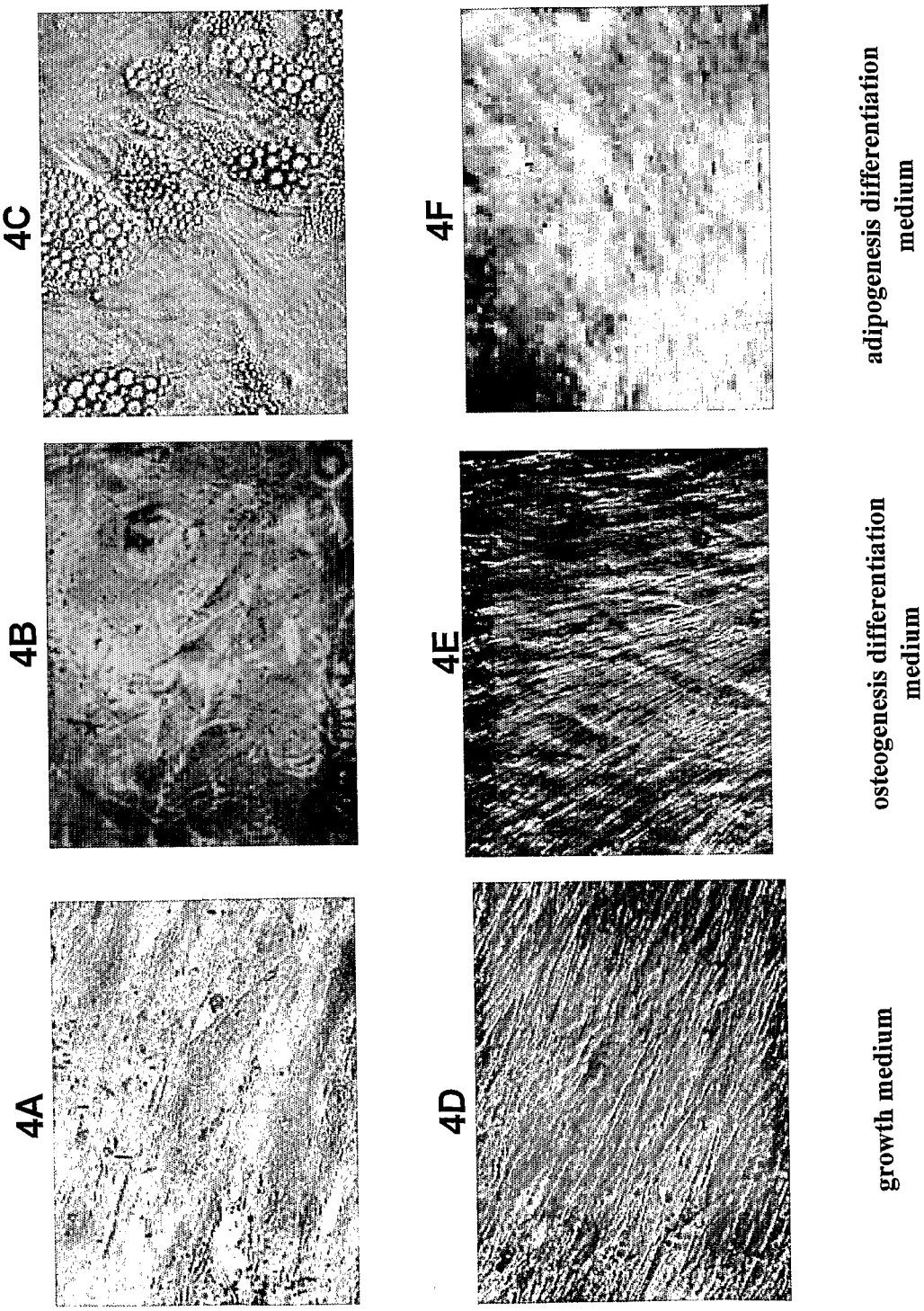

% expressing cells
120.00
100.00
80.00
60.00
40.00
20.00
0.00
CD200
CD11b
CD34
HLA-DR
CD14
CD19
CD45
CD29
CD73
CD90
CD105
Marker

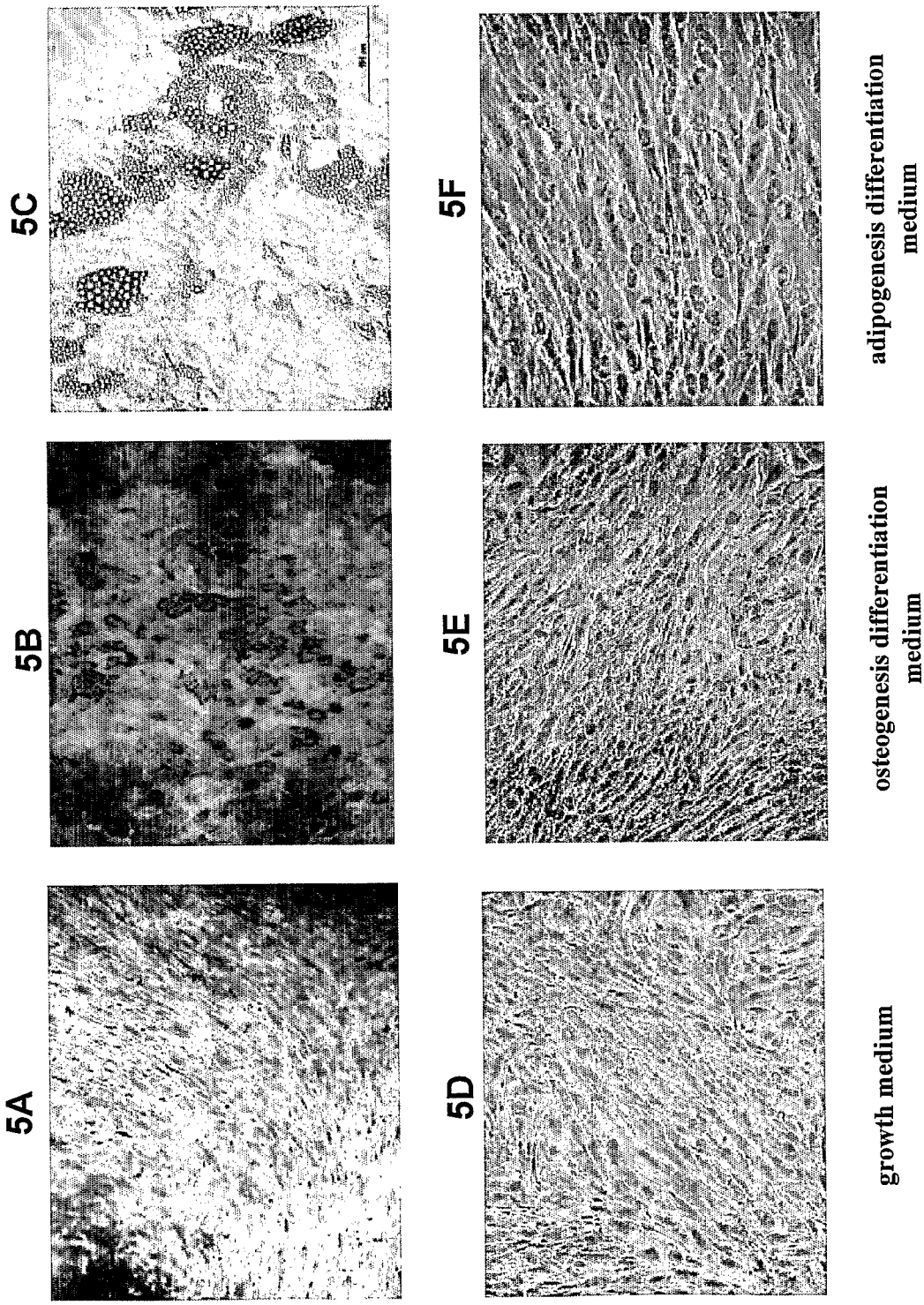
FIG. 3


Radioactivity
(CPM)
70000
60000
50000
40000
30000
20000
10000
0
MNCs w/o PHA
MNCs
PLC28
PLC30
P030308
P190508A

FIGs. 4A-F


4A
4B
4C
4D
4E
4F
growth medium
osteogenesis differentiation medium
adipogenesis differentiation medium

FIGs. 5A-F


5A
5B
5C
5D
5E
5F
growth medium
osteogenesis differentiation medium
adipogenesis differentiation medium