(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 July 2009 (09.07.2009)

PCT

(10) International Publication Number
WO 2009/085969 A2

(51) International Patent Classification:
C12N 5/08 (2006.01) *C12N 5/00* (2006.01)
C12N 5/02 (2006.01)

(21) International Application Number:
PCT/US2008/087452

(22) International Filing Date:
18 December 2008 (18.12.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/014,987 19 December 2007 (19.12.2007) US

(71) Applicant *(for all designated States except US)*: **REGENERATIVE SCIENCES, LLC** [US/US]; 6850 West 116th Ave, Unit D, Broomfield, CO 80020 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **CENTENO, Christopher, J.** [US/US]; 6850 West 116th Ave., Unit D, Broomfiield, CO 80020 (US).

(74) Agents: **SWANSON & BRATSCHUN, L.L.C.** et al.; 8210 SouthPark Terrace, Littleton, CO 80120 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

WO 2009/085969 A2

(54) **Title:** COMPOSITIONS AND METHODS TO PROMOTE IMPLANTATION AND ENGRAFMENT OF STEM CELLS

(57) **Abstract:** Tissue repair in-vivo depends on acute inflammation, but in many clinical situations the other major components of healing such as blood supply, anabolic hormones, growth factors, and stem cells are lacking. This invention includes compositions consisting of an agent which induces an inflammatory healing response combined with an autologous platelet lysate at a specific concentration which may have demonstrated in-vitro abilities to expand autologous tissue repair cells.

COMPOSITIONS AND METHODS TO PROMOTE IMPLANTATION AND ENGRAFMENT OF STEM CELLS

TECHNICAL FIELD

[0001] The invention generally relates to compositions and methods for induction of tissue repair in a patient in need thereof. More particularly, the invention relates to compositions and methods for inducing diffuse micro-tissue injury and enhanced cell growth at a site to facilitate in-*vivo* tissue repair and/or replacement in a patient in need thereof.

BACKGROUND OF THE INVENTION

[0002] Mesenchymal stem cells (MSCs) are pluripotent blast or embryonic-like cells located in blood, bone marrow, dermis and periosteum. In general these cells are capable of renewing themselves over extended periods of time as well as, under various environmental conditions, differentiating into cartilage, bone and other connective tissue. Recently, various investigators have researched the potential for using these cells to repair or regenerate target tissues, *e.g.*, bone, cartilage, cardiac muscle, etc. In this manner MSCs have been reported to have regenerative capabilities in a number of animal models. See Acosta et al. (2005) Neurosurg Focus 19(3):E4; Barry (2003) Novartis Found Symp. 249:86-102, 170-4, 239-41; Brisby et al. (2004) Orthop Clin. North Am. 35(1):85-89; Buckwalter and Mankin (1998) Instr Course Lect. 47:487-504; Caplan (1991) J Orthop Res. 9(5):641-650.

[0003] Recent research has shown that tissue injury can act as a homing signal for bone marrow derived MSCs to migrate to the site of injury. (Agung et al., Knee Surg. Sports Traumatol Arthrosc, 2006, 14(12):1307-14). However, these studies utilized a surgical approach to include a gross tissue injury which was shown to signal MSCs to the injury site, this approach is, however, likely impractical for clinical care (for example cutting portions of an ACL ligament to signal MSC homing to the ACL site could result in more damage than actual repair to the ACL). Note also that other researchers have discussed the possibility that tissue injury can act as a homing signal for MSCs into various tissues. (Ramirez et al., Br J Sports Med., 2006 40(8):719-22; Shyu et al., Front Biosci., 2006 11:899-907).

[0004] In addition, injectable hyperosmolar substances that initiate tissue injury and potentially prompt healing in a clinical setting have been utilized to varying success. (Centeno et al., Pain Physician, 2005, 8(1):67-72; Mooney, V., Spine J, 2003 3(4):253-4; Reeves et al., J Altern Complement Med., 2000 6(4):311-20; and Reeves et al., Altern Ther Health Med 2000 6(2):68-74). However, these procedures have had limited practical success in the health care setting.

[0005] Clinical advantage could be gained through minimally invasive medical procedures that impart stem cells to a site of need within a patient (for example, percutaneous injection of MSCs to a site in need). Unfortunately, mere implantation of stem cells to a site in this manner has proven mostly ineffective. As such, there is a need in the health care setting to more optimally utilize stem cell implants as well as to facilitate repair of sites in a patient without first grossly injuring the site to initiate a repair process.

[0006] Against this backdrop the present invention was developed.

SUMMARY OF THE INVENTION

[0007] The present invention provides repair compositions for facilitating tissue repair and/or replacement in a patient in need thereof. Repair compositions include an effective amount of a cell growth enhancing composition in combination with one or more inflammation inducing agent(s).

[0008] Aspects of the cell growth enhancing composition include the use of autologous and/or non-autogous cell growth enhancing materials. Cell growth enhancing compositions can include an autologous composition spiked with one or more non-autologous factors. Typical autologous cell growth enhancing compositions include platelet rich fibrin solutions, *e.g.,* 5% to 40% platelet lysate solutions, and/or platelet gels. Typical non-autologous growth compositions include recombinant growth factors such as insulin-like growth factor.

[0009] Aspects of the inflammation inducing agents include agents that induce local micro-diffuse injury at the site in need of repair and include osmolar agents, inflammatory cytokines, and/or sclerosing agents. In some aspects combinations of these agents can be utilized, for example a combination of osmolar agents with a sclerosing agent.

[0010] Repair compositions of the invention can further include essential nutrients useful for the site in need of repair, for example collagen where the repair site is a knee joint in need of

cartilage repair. In addition, repair compositions can include anabolic hormones, like human growth hormone, for further tissue growth signaling in the repair site.

[0011] Finally, repair compositions of the invention can include stem cells and in particular isolated stem cells, for example, isolated autologous or non-autologous mesenchymal stem cells. Stem cells can be delivered to the site in the repair composition or separately from the repair composition, *i.e.,* both separated physically and temporally.

[0012] The present invention also provides methods for facilitating tissue repair in a patient in need thereof. Methods include harvesting and preparing a repair composition, *e.g.,* a 5% to 40% platelet lysate from a patient having a repair site in need of treatment and an inflammation inducing agent; administering to the repair site the repair composition (in an amount necessary to endure and maintain repair) in accordance with embodiments of the invention described herein; and optionally administering stem or other like repair cells to the repair site to facilitate tissue repair at the site in need thereof. In some aspects of the methods herein, the repair composition is a first inflammation inducing agent composition and a second cell growth enhancing composition, where the first composition is administered to the repair site to induce local micro-tissue injury followed by administration of the second composition to enhance cell growth at the same site. In some instances, stem cells or other repair-like cells are administered to the site to enhance the repair process, typically with or shortly after administration of the cell growth enhancing composition. Methods herein can include multiple applications of repair compositions over the course of 1 week to 6 months.

[0013] Finally, the present invention provides pharmaceutical compositions for use in therapeutic applications. In some cases the pharmaceutical compositions are used to treat a patient with a site of injury in need of repair and in some cases the patient has osteoarthritis, osteoporosis or other like degenerative disease.

[0014] These and various other features and advantages of the invention will be apparent from a reading of the following detailed description and a review of the appended claims.

DETAILED DESCRIPTION OF THE INVENTION

[0015] Embodiments of the present invention provide repair compositions for facilitating tissue repair and/or tissue replacement in a patient in need thereof. For purposes herein a patient refers to any mammal and preferably human having a need for the compositions and/or methods

of the present invention. In one embodiment, repair compositions include a therapeutically effective amount of a cell growth enhancing composition in combination with one or more inflammation inducing agents.

[0016] Embodiments of the invention include repair compositions where the cell growth enhancing composition is an autologous or non-autologous growth factor(s), including, for example, recombinant growth factors. In other embodiments the cell growth enhancing composition is a mixture of autologous and non-autologous growth factor(s).

[0017] In typical embodiments the cell growth enhancing composition is one or more autologous growth factor(s) from the patient in need thereof, *i.e.*, the patient having the site of injury in need of repair. Cell growth enhancing growth factor can include autologous platelet and/or platelet lysate composition(s).

[0018] Embodiments of the invention further include repair compositions where the inflammation inducing agent(s) is an agent capable of inducing micro-tissue or localized injury at the site where tissue repair is required. Inflammation inducing agents for use herein include osmolar agents, inflammatory cytokines, sclerosing agents, and the like. As such, a repair composition can include an autologous platelet lysate combined with one or more inflammation inducing agents.

[0019] Repair compositions of the present invention can be administered through a surgical incision, arthroscopically and/or percutaneously. A site of repair in a patient for purpose of the present invention is any site in need of tissue repair or re-growth, for example a knee in need of cartilage, a liver in need of hepatocytes, a bone in need of osteocytes, etc.

[0020] Embodiments of the present invention also provide methods for facilitating tissue repair in a patient in need thereof. Methods include administering a repair composition that includes both the cell growth enhancing composition and one or more inflammation inducing agent(s); or administering non-contemporaneously one or more inflammatory inducing agent(s) and a cell growth enhancing composition.

[0021] In one embodiment, methods include obtaining an autologous growth enhancing composition from the patient in need of tissue repair; administering an inflammatory inducing agent to the patient in an amount sufficient to induce local inflammation at the site in need of tissue repair; and administering the autologous growth enhancing composition to the patient at the site in need of tissue repair in an amount to effectively facilitate cell growth/expansion at the

site. In some embodiments the inflammatory inducing agent and growth enhancing composition are administered contemporaneously via separate compositions; in other embodiments the inflammatory inducing agent and growth enhancing composition are administered within 24 to 96 hours, more preferably between 72 and 96 hours, of each other. Multiple administrations can be performed over the course of 1 to 6 months (or more dependent on health professionals determination). In other embodiments the inflammatory inducing agent and growth enhancing composition are combined and administered as one composition.

Definitions:

[0022] The following definitions are provided to facilitate understanding of certain terms used frequently herein and are not meant to limit the scope of the present disclosure.

[0023] "Cell growth enhancing composition" refers to growth factors like recombinant FGF, recombinant TGF-beta, autologous compositions like platelets, platelet rich fibrin, platelet rich plasma, platelet lysate, platelet gels, and the like and can include growth factors, cytokines, hormones, essential nutrients or other proteins, fatty acids, or carbohydrates.

[0024] "Inflammation inducing agent" refers to any agent capable of inducing diffuse micro-tissue injury at a site, including osmolar agents like hypertonic dextrose, inflammatory cytokines, *e.g.*, MIP-1, MIP-1α, MIP-1β, and MIP-2, sodium morrhuate, pumice and phenol.

[0025] "Mesenchymal stem cell" or "MSCs" refers to multipotent stem cells capable of differentiating into osteoblasts, chondrocytes, myocytes, adipocytes, neuronal cells, pancreatic islet cells, and the like (see below). Source MSCs of the invention are typically harvested from the iliac crest of the patient in need of the repair (or a suitable donor, (non-autologous)), such patient is referred to herein as a "patient in need thereof" (note that other sources, such as adipose tissue, synovial tissue, and connective tissue have recently been identified and are also considered as MSC sources within the scope of the present invention). In one embodiment, approximately 10-20 cc of bone marrow is harvested and "isolated" using methods described in US Patent Application 60/761,441 to Centeno or through adherence to plastic, as described in US Patent No. 5,486,359 to Caplan et al. Each of these references is incorporated herein in their entirety for all purposes.

[0026] "Platelet lysate" refers to the combination of natural growth factors contained in platelets that has been released through lysing of the platelets. This can be accomplished through

chemical means (*i.e.* $CaCl_2$), osmotic means (use of distilled H_2O), or through freezing/thawing procedures. Platelet lysates of the invention can also be derived from whole blood and can be prepared as described in US Patent No. 5,198,357, which is incorporated by reference herein. Alternatively, platelet lysate for use herein can be prepared from a bone marrow harvest using the method of Doucet (*Doucet, Ernou et al.,* 2005, Journal of Cellular Physiology, 205(2):228-236), which is incorporated by reference herein in its entirety). Typical lysates include from about tens of millions to 100's of billions platelets. As shown by *Martineau et al.*, Biomaterials, 2004 25(18) p4489-503 (incorporated herein by reference in its entirety), platelet lysates inherently include the growth factors required to facilitate consistent MSC growth. In typical embodiments the platelet lysate is autologous and is in an amount useful for effective and consistent use in embodiments herein. In particular, it should be noted that while the levels of growth factors such as TGF-beta are much lower in platelet lysate than those commonly used in vitro to expand MSCs, it is believed that there are significant synergistic effects when all of the low level growth factors contained in platelet lysate are used together.

[0027] "Protein," "peptide," and "polypeptide" are used interchangeably to denote an amino acid polymer or a set of two or more interacting or bound amino acid polymers.

[0028] "Stem cells" refers to any cell having the characteristic of being unspecialized and able to renew for extended periods of time through cell division and being inducible to become cells with specialized function.

Tissue Repair Compositions of the Invention

[0029] Compositions of the invention include tissue repair compositions having enhanced capacity for tissue repair and/or replacement in a patient in need thereof. Compositions typically include two different aspects, a first aspect is directed toward induction of a local inflammatory response at the site where tissue repair is required (in some cases due to cell lysis caused by the inflammatory agent(s)); and a second aspect directed at facilitating cell growth (autologous or non-autologous cell growth enhancing materials) at the same site. The combination of inflammation and cell growth induction is more impressive and unexpected than convention tissue repair methodologies. In some embodiments a third aspect is included, autologous or non-autologous stem cells for facilitating the repair composition capacity to repair or replace tissue at a site in need.

[0030] Typical repair compositions herein include combinations of at least one or more inflammation inducing agent(s) with at least one or more cell growth enhancing composition(s). In one embodiment the cell growth enhancing composition(s) for use herein can include one or more autologous factor(s). In another embodiment the cell growth enhancing compositions(s) for use herein can include one or more non-autologous factor(s). In other embodiments the repair compositions include a combination of autologous and non-autologous growth factors.

[0031] Typical autologous growth factors used herein include: platelets, platelet rich plasma, platelet rich fibrin, platelet lysate, or mixtures thereof.

[0032] As described herein, typical non-autologous factors include recombinant growth factors, *e.g.*, epidermal growth factor, fibroblast growth factor-2, vascular endothelial growth factor, insulin-like growth factor, transforming growth factor-β and platelet-derived growth factor. Recombinant growth factors can be purchased from various manufacturers (*e.g.*, RDJ, Inc., Bio Vision Inc., Bio Clone Inc., etc.) or through known isolation and purification techniques.

[0033] In addition, repair compositions of the invention can include autologous growth factor compositions enriched with recombinant growth factors, for example, a platelet lysate prepared from the patient in need of tissue repair spiked with recombinant transforming growth factor-β.

[0034] Embodiments herein can include repair compositions having one or more inflammation inducing agents. Inflammatory inducing agents as defined herein are agents that induce local cell injury, in some cases the inflammation inducing agent is hypertoxic dextrose, sodium morrhuate, pumice, phenol, and/or one or more inflammation inducing cytokine(s). Inflammation inducing cytokines for use herein include macrophage inflammation protein-1 (MIP-1), MIP-1α, MIP-1β, and MIP-2.

[0035] In one embodiment, a patient is treated with a repair composition that includes 5-50% hypertonic dextrose. In a second embodiment, a patient is treated with a repair composition that includes a dose of 1%-10% sodium morrhuate. Finally, repair compositions can include phenol and be used in a patient in need thereof at a dose of from about 1%-20%. The total volume of this aspect of the invention can be variable but can be from 1 to 5 milliliters per administration of the agent.

[0036] In addition, inflammation inducing agents of the invention can include materials that exacerbate a local injury and thereby increase the effectiveness of the repair compositions of the invention. Materials used herein include gels, hydrogels, and foams. In some cases the gels, hydrogels and/or foams are bioabsorbable. These high density mixtures can then be diluted by the body's own repair response or can be diluted back to the 0.9% physiologic range by a subsequent treatment of normal saline. Thus, for example, repair compositions can include agents that exacerbate local injury (gels, hydrogels, foams) which may be combined with other inflammation inducing agents, including hypertoxic dextrose, sodium morrhuate, phenol and the like.

[0037] In typical embodiments the repair composition includes a cell growth enhancing composition of an autologous growth factor, for example a platelet lysate. A platelet lysate between from about 5% to about 40% and more typically between 5% and 20% is preferred, although other concentrations can be used. Platelet lysate solutions can be obtained and prepared according to the methods and compositions as described in US Patent Application 11/773,774, which is incorporated herein by reference for all purposes (other methodologies have been discussed previously). Total volume of prepared platelet lysate administered to a patient can be from 1ml to 40 ml and in some cases 1ml to 20 ml.

[0038] One problem with clinical use of platelet lysate in a patient is the variability in the bioavailability and concentrations of growth factors in the particular platelet lysate. As a result, without specific biological assays to determine factor levels in the lysate, dosing lysate becomes difficult. Research discussed in US Patent Application 11/773,774 clearly showed that some patients yielded maximum possible *in-vitro* expansion in 5% lysate, while others required up to a 400% increase in concentration of PL to achieve maximum expansion. Even if assays of growth factors were clinically available and commonly used, the bioavailablility of these growth factors would still be difficult to access.

[0039] In this case, the availability of culture expansion data with this patient's platelet lysate provides data about the activity of these growth factors (as discussed in US Patent Application 11/773,774 and incorporated by reference). This data can be used to identify the optimal platelet lysate % for use in a target patient, *i.e.,* culture autologous MSCs with variable amounts of autologous platelet lysate.

[0040] Repair compositions of the invention can further include essential nutrients to further enhance tissue repair, for example collagen, glycoaminosglycan's, amino acids, peptides, proteins, sodium pyruvate, glucose, glutamine, ribonucleosides, deoxyribonucleosides, carbohydrates, essential oils, and the like. As such, a platelet lysate solution can be spiked with collagen and various amino acids to facilitate the repair process in the patient.

[0041] Repair compositions of the invention can also include anabolic hormones, for example, human growth hormone, testosterone, and the like. Again, for example, a platelet lysate could be spiked with a target anabolic hormone prior to administering to the patient in need thereof.

[0042] Finally, repair compositions as described herein can include autologous or non-autologous stem cells to enhance repair and re-growth of the repair site. In one embodiment, mesenchymal stem cells (MSCs) are prepared and expanded in accordance with US Patent Application 11/773,774, (incorporated by reference previously), and implanted to the repair site. Note that other stem cell or cell types are within the scope of the present invention, however, MSCs are identified as one potential embodiment herein.

[0043] Recently, Centeno et al. (US Patent Application 11/773,774) described a method for expanding MSCs using a growth channel and autologous platelet lysate. Also described were methods for transplanting certain levels of growth factors (platelet lysate or platelets) with the expanded MSCs to the area in a patient in need of repair. The levels of these growth factors were based on a percentage of platelet lysate needed to optimally expand certain cells ex-vivo. These techniques can also be utilized to provide a sufficient number of MSCs for administration to the patient in need.

Method of Facilitating Tissue Repair in a Patient in Need Thereof:

[0044] Embodiments described herein include methods for the therapeutic restoration of a site in a patient in need thereof. For example, therapeutic restoration of a degenerative disc or cartilage of a joint in need thereof. Other examples include the replacement of cardiac muscle in the heart.

[0045] Methods herein include initially determining parameters for optimally treating a patient's repair site. For example, a determination of what and how much inflammation inducing agent(s) would work best at the site of injury as well as to determine what and how much cell

enhancing growth composition should be used (autologous, non-autologous, mixture, etc). In this regard, the site should have enough micro injury to direct cellular repair mechanisms without causing more macro injury to the site which is incapable of healing. In addition, a determination on whether a repair composition of the invention would be used, or whether an inflammation inducing agent composition would be used initially followed by contact with a cell growth enhancing composition. Administration of the repair composition to the site of repair in the patient is then followed by injury site analysis.

Therapeutic Applications

[0046] Repair compositions of the invention provide optimal repair conditions/environment to a repair site in a patient in need thereof. Repair compositions both induce micro-tissue injury, thereby signaling the patient's inflammatory factors and additionally initiate and/or facilitate cell growth at the site. In some embodiments, ex-vivo cultured stem cells are implanted into the environment to further increase the potential success of repair at the repair site.

[0047] Repair compositions herein can be formulated as pharmaceutical compositions and administered to a patient in need thereof, preferably a mammalian host, including a human patient. Repair compositions can be formulated in a variety of forms adapted to the chosen route of administration.

[0048] Embodiments herein include repair compositions that include a pharmaceutically acceptable carrier and/or specific delivery drug.

[0049] For administration of the composition as an injectable solution or suspension, repair compostitions can be formulated according to techniques well-known in the art, using suitable dispersing or wetting and suspersing agents , such as sterile oils, including synthetic mono- or diglycerides, and fatty acids, including oleic acid.

[0050] Soutions or suspensions of the repair compositions can be prepared in water, isotonic saline (PBS), and optionally mixed with a nontoxic surfactant. Dispersions may also be prepared in glycerol, liquid polyethylene, glycols, vegetable oils, triacetin and mixtures thereof. Under customary use and storage conditions, the repair compositions herein may contain one or more preservatives to prevent growth of microorganisms.

[0051] Therapeutic applications herein, refers to use of the compositions and methods of the invention to treat a patient having a site of injury in need of tissue repair. Sites of injury in need or repair, *i.e.,* repair sites, include joints in need or cartilage repair and/or regrowth, bone in need of bone repair or regrowth, tendon/ligament in need of repair or regrowth, organ repair in need of functional cell repair and/or regrowth (for example, cardiac muscle growth in a heart), and the like.

[0052] In some embodiments, the invention is directed at therapeutic applications for patients having disease states that limit their inherent ability to repair or re-grow cells in a repair site. For example, patient's having osteoarthritis, osteoporosis, avascular necrosis, would all benefit from the facilitated repair compositions and methods of the present invention.

[0053] This invention focuses on creating conditions that mimic inflammation to induce tissue repair. Most animal research in this arena has been performed in acute injury models (meaning an injury is experimentally created and is still acute or sub-acute when MSCs are introduced to promote tissue repair). This is a poor surrogate for a chronic osteoarthritis model where no acute injury exists. The inventor's research in this area has shown that the creation of an acute osmolar micro injury can assist in MSC related meniscus repair (see Example 1). In these cases we used percutaneously delivered hypertonic dextrose to initiate an injury and followed this with the percutaneous delivery of culture expanded MSCs (expansion carried out per US Patent Application 11/773,774 incorporated by reference for this purpose).

[0054] Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

EXAMPLES

EXAMPLE 1: Therapeutic use of Embodiments of the Present Invention

[0055] Approximately 20 ml of whole bone marrow was extracted from two patients, CD (40 year old, white male) and JV (28 year old, white male). CD held a pre-op diagnosis of severe osteoarthritis of the knee with evidence of myxoid degeneration of the medial>lateral meniscus and JV held the pre-op diagnosis of a chronic bucket handle tear of the posterior horn of the medial meniscus.

[0056] Each patient was then placed prone on an OR table and the area to be harvested was numbed with 1% Lidocaine, and a sterile disposable trocar was used to draw 10 cc of marrow blood from the right PSIS area and 10cc from the left PSIS area.

[0057] Whole marrow was centrifuged at 100g for 4-6 minutes to separate the plasma from the RBCs. The plasma was removed, placed in a separate tube, and centrifuged at 1000g for 10 minutes to pellet the nucleated cell fraction. The nucleated cells were washed once in PBS, counted, and then resuspended in DMEM + 10% platelet lysate (PL) and seeded at $1x10^6$ cells/cm^2 in monolayer flask culture. Cultures were incubated at 37°C/5% CO_2 in a humidified environment. The culture medium was changed after 3 days, removing the majority of the non-adherent cell population. MSC colonies developed 6-12 days after seeding. After growing to near confluence, the colonies were trypsinized over 30-60 seconds such that only the colony-forming MSCs detached. The MSCs were reseeded at a density of 12,000 cells/cm^2 in DMEM + 5%, 10%, or 20% PL. Each culture was passaged 1:3 after reaching 40-50% confluence.
After MSCs had been grown to the 3^{rd}-5^{th} passage, they were suspended in phosphate buffered saline (PBS). The patient was brought back to clinic and was consented in writing.

[0058] The following operative course was taken:

1. Each patient was first treated with 12.5% dextrose (hyper-osmolar agent) and local anesthetic injected intra-articular via c-arm through a medial inferior port of the involved knee.

2. 3-5 days later, after the acute inflammatory response had subsided from the initial injection, culture expanded autologous MSCs in PBS were injected with 10% platelet lysate.

[0059] Modified VAS questionnaires and Functional Rating Index questionnaires were provided to the patient and administered before the procedure, 1 month after the procedure, and three months after the procedure. Range of motion measurements of the knee were measured by a physical therapist before the procedure, 1 month post-procedure and 3 months post procedure. In addition, pre-procedure MRI's were obtained on a GE 3.0 T magnet with Proton Density Fast

Spin sequences in the sagittal coronal planes. Post procedure images at 1 month and at 3 months were obtained using mating excitation times (NEX), repetition times (TR), and echo times (TE). Quantitative meniscus and articular cartilage volume analysis was carried out using commercially available image processing software (OSIRIS- Digital Imaging Unit, Division of Medical Informatics, University Hospital of Geneva) using three traces by the same examiner of each region of interest (ROI). Standard deviation from the mean was calculated for these three traces. The area of the medial weight bearing femoral defect was also traced and calculated in a similar manner.

Results (see Table 1):

Table 1: MRI volume changes in femoral cartilage and meniscus from pre-procedure, 1 month post procedure, 3 months post procedure, and 6 months post procedure:

Patient Name	Joint	Time	Area of Measurement	Volume e (n=3)	STDEV	SE	Cell # Injected (millions)	% Change from Pre-injection
1.	L Knee	Pre-injection	Cartilage surface meniscus	4535 2646	215.37 126.05	124.49 72.86	32.66	
		1 month	Cartilage surface meniscus	5484 3233	128.34 95.35	74.19 55.11		20.93 22.18
		3 months	Cartilage surface meniscus	4867 2979	378.02 154.44	218.51 89.27		7.32 12.59
		6 months	Cartilage surface meniscus	5531 4055	120.97 168.57	69.93 97.44		21.96 53.25
2.	R Knee	Pre-injection	Cartilage surface meniscus	7994 2512	113.51 178.5	65.61 103.18	20.9	
		1 month	Cartilage surface meniscus	8150 2632	131.04 126.65	75.75 73.21		1.95 4.78
		3 months	Cartilage surface meniscus	9121 3322	468.93 246.55	271.06 142.51		14.10 32.25

[0060] The results from the example show the surprising effectiveness of embodiments of the invention and the utility of using compositions and methods in accordance with the present invention.

EXAMPLE 2: MSCs Expand in Presence of Dextrose

[0061] To ensure that the various growth factors commonly found in platelet lysate (TGF-beta, FGF, IGF, PDGF) could be exposed to a hypertonic environment and still function to support mesenchymal stem cell growth, the following experiment was carried out with culture expanded human MSCs:

Method:

[0062] To 0.8mL of 10% platelet lysate was added 0.2mL of 50% Dextrose. In a separate condition, to 0.8ml of 10% platelet lysate (PL) we added 0.2mL of Phosphate Buffered Saline. We allowed the two samples to incubate 1hr at 37C in a 5% CO2 environment. 1 mL of each suspension was then removed and added to 9 mls of basic alpha mem media to get final ratio of 10%PL and 1% Dextrose. Each well of a 6 well plate was then seeded with 100,000 cells in each suspension. After 48hr incubation all of the cells appeared morphologically normal.

Results:
PL + = with Dextrose
PL - = control

PL +	PL -	Average	Std Dev	CV
2.50E+05	1.90E+05	2.20E+05	30000	13.636

The example shows that MSCs can be effectively expanded in the presence of dextrose (inflammatory inducing agent), a surprising and unexpected result.

Claims:

What is claimed is:

1. A composition for facilitating tissue repair in a patient in need thereof comprising a therapeutically effective amount of an autologous platelet lysate in combination with one or more inflammation inducing agent.
2. The composition of claim 1, wherein the inflammation inducing agent is an agent capable of inducing local cell injury, in the patient in need thereof.
3. The composition of claim 2, wherein the agent for inducing local cell injury is selected from the group consisting of hypertonic dextrose, sodium morrhuate, inflammatory cytokines, pumice, phenol and mixtures thereof.
4. The composition of claim 2, wherein the agent for inducing local cell injury acts through local cell lysis via a hyper or hypo osmolar agent.
5. The composition of claim 1, wherein platelet rich plasma, platelet rich fibrin, or another whole platelet concentrate is substituted for platelet lysate.
6. The composition of claim 1, wherein cytokines are added to the 5% to 40% platelet lysate to alter the timing of growth factor deganulation off of platelets in the platelet lysate.
7. The composition of claim 1, further comprising one or more essential nutrients wherein the essential nutrients further facilitate tissue repair.
8. The composition of claim 7, wherein the one or more essential nutrient is selected from the group consisting of glycoaminosglycan's, collagen, amino acids, peptides, proteins, sodium pyruvate, glucose, glutamine, ribonucleosides, deoxyribonucleosides, carbohydrates, and essential oils.
9. The composition of claim 1, further comprising one or more anabolic hormones added to amplify local healing effects.
10. The composition of claim 1, wherein the inflammation inducing agent further comprises a gel, hydrogel, foam, or other material to localize or maximize the micro-injury to an area of application.
11. A method for facilitated tissue repair in a subject in need thereof comprising:

    obtaining an amount of platelets from the subject;

    preparing a 5% to 40% platelet lysate solution from the platelets;

administering an inflammation inducing agent to the subject in an amount sufficient to induce local inflammation at the site in need of tissue repair; and administering the 5% to 40% platelet lysate solution to the subject at the site in need of tissue repair;

wherein the combined administration of the inflammation inducing agent and platelet lysate facilitates tissue repair at the site in the subject in need of tissue repair.

12. The method of claim 11, further comprising determining the % platelet lysate for administering to the patient by first determining what % of platelet lysate is necessary to optimally culture expand the patient's mesenchymal stem cells in vitro and using this same % platelet lysate for administering to the patient.

13. The method of claim 11, where administering to the patient is to the site in need of repair through a surgical incision, arthroscopically or percutaneously.

14. The method of claim 11, wherein the % platelet lysate and the amount administered, is determined based on at least the patient's age, health, repair site, and level of repair required.

15. A composition for facilitating tissue repair in a patient in need thereof comprising a therapeutically effective amount of one or more recombinant growth factors in combination with one or more inflammation inducing agent(s).

16. The composition of claim 15, wherein the inflammation inducing agent is selected from the group consisting of hypertoxic dextrose, sodium morrhuate, inflammatory cytokines, phenol and mixtures thereof.

17. The composition of claim 15, further comprising 5% to 40% platelet lysate wherein the platelet lysate is prepared from whole blood harvested from the patient in need of tissue repair.