



# Regeneration of meniscus cartilage in a knee treated with percutaneously implanted autologous mesenchymal stem cells <sup>☆</sup>

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**Summary** Mesenchymal stem cells are pluripotent cells found in multiple human tissues including bone marrow, synovial tissues, and adipose tissues. They have been shown to differentiate into bone, cartilage, muscle, and adipose tissue and represent a possible promising new therapy in regenerative medicine. Because of their multi-potent capabilities, mesenchymal stem cell (MSC) lineages have been used successfully in animal models to regenerate articular cartilage and in human models to regenerate bone.

The regeneration of articular cartilage via percutaneous introduction of mesenchymal stem cells (MSC's) is a topic of significant scientific and therapeutic interest. Current treatment for cartilage damage in osteoarthritis focuses on surgical interventions such as arthroscopic debridement, microfracture, and cartilage grafting/transplant. These procedures have proven to be less effective than hoped, are invasive, and often entail a prolonged recovery time.

We hypothesize that autologous mesenchymal stem cells can be harvested from the iliac crest, expanded using the patient's own growth factors from platelet lysate, then successfully implanted to increase cartilage volume in an adult human knee.

We present a review highlighting the developments in cellular and regenerative medicine in the arena mesenchymal stem cell therapy, as well as a case of successful harvest, expansion, and transplant of autologous mesenchymal stem cells into an adult human knee that resulted in an increase in meniscal cartilage volume.

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Regenerative medicine can be broken into three main areas: platelet augmentation, stem cell isolates, and recombinant growth factor amplification. These new technologies have used either alone or can be combined. For example, adult stem cells require various growth factors to maintain their growth and engraftment [1–5]. As a result, stem cells are usually transplanted with growth factors.

## Autologous platelet approaches

The platelet augmentation approach is based on the concept that platelets contain key growth factors such as platelet derived growth factors (PDGF's), transforming growth factors (TGF's), fibroblast growth factors (FGF's), and various forms of interleukins (IL) [6]. In response to tissue injury, a complex cascade of cellular and noncellular signals triggers platelet receptors, resulting in expulsion of these growth factors within the site of injury. This process is known as degranulation and initiates cellular proliferation and a tissue repair response. One of the key triggers for platelet activation is the protein thrombin, which has been shown to induce immediate platelet growth factor release in vitro in a dose dependant fashion [7].

Understanding platelet physiology has led to the concept of utilizing platelet growth factors to develop novel and natural regenerative therapies. Several commercially available centrifugation systems can isolate and concentrate platelets from an autologous blood sample. The resulting blood isolate is known as platelet rich plasma (PRP) which contains a high concentration of stored autologous growth factors. Red blood cells are separated from PRP which typically contains a hematocrit below 5%. Most commercially available PRP centrifuge products have the ability to produce a 4–7-fold increase in platelet concentration compared to whole blood.

By exposing PRP to thrombin, platelet degranulation is induced and a concentrated pool of autologous growth factors in physiologic combinations can be delivered to injured tissue as a therapeutic modality to augment natural regenerative pathways. Once activated by thrombin, PRP is termed platelet gel (PG), due to initiation of clotting pathways mediated by fibrin in the serum. Initial PRP studies used bovine thrombin as an activator. It has since been discovered that bovine thrombin can lead to development of antibodies to clotting factors V, XI and autologous thrombin. In rare case reports, this has lead to systemic and life threaten-

ing coagulaopathies with multisystem failure [8]. New techniques have since been developed to generate autologous thrombin from the same blood sample used to make PRP, with superior properties to bovine thrombin.

Percutaneously, PRP can be implanted into target tissue along with autologous thrombin via a double lumen injection technique. Alternatively, PRP can be activated by thrombin in vitro to produce a lower viscosity platelet lysate (PL) containing growth factors without platelet tissue or a fibrin matrix. PL has been successfully used as a cell culture additive to facilitate growth and differentiation of autologous mesenchymal stem cells [3,9–11].

Sound basic science and animal testing data, along with a handful of human clinical trials have demonstrated a strong safety profile and encouraging therapeutic results. Mishra has investigated PRP injections into chronic elbow tendonosis, reporting greater than 90% reduction in pain up to two year out from the intervention [12]. Aspenberg and Virchenko found that a single PRP injection into a transected rat Achilles tendon increased tissue strength by 30%, with histology showing greater maturation of tendon cells vs. control [13]. Based on work by Anitua, the application of PG therapy has broad applications within orthopedic medicine. He hypothesizes that the released GF's have a chemotactic and mitogenic effect on mesenchymal stem cells (MSC's) and osteoblasts when applied to bony tissues [8]. This author has also hypothesized other mechanisms of action in addition to recruitment of progenitor cells to replace damaged tissue, include angiogenesis, local anabolic stimulation of cellular protein synthesis and gene expression, leading to proliferation, tissue remodeling and production of new extracellular matrix (ECM).

## Stem cells and regenerative medicine

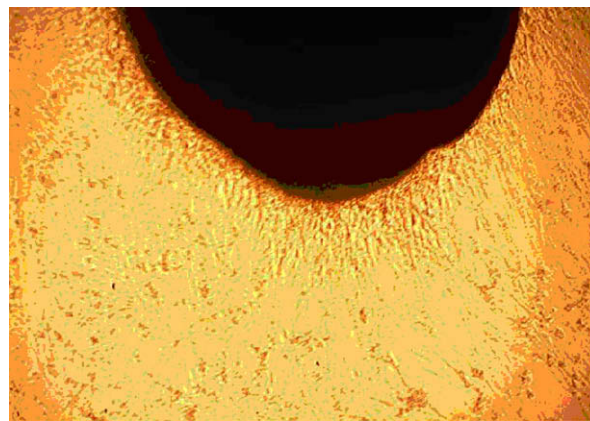
Embryonic stem cells have been in the popular media for many years and have shown promise scientifically while creating significant controversy. As a result, many researchers have focused on adult stem cells, or stem cells isolated from adult humans that can be transplanted into damaged tissue. Adult stem cells consist of three main types: CD34+ heme progenitors destined to become blood components, endodermal stem cells used to replace endodermal linings such as endometrium, and mesenchymal stem cells (MSC's). MSC's are pluripotent cells found in multiple human adult tissues including bone marrow, synovial tissues, and adipose tissues. Since they are derived from the

mesoderm, they have been shown to differentiate into bone, cartilage, muscle, and adipose tissue [14]. Because of their multi-potent capabilities, mesenchymal stem cell (MSC) lineages have been used successfully in animal models to regenerate articular cartilage and in human models to regenerate bone [15–28]. Of importance to the interventional pain management community is recent research demonstrating that articular cartilage may be able to be repaired via percutaneous introduction of mesenchymal stem cells (MSC's) [28–30]. Research into MSC's has exploded in recent years. As an example, a PubMed search for the year 1999 reveals about 90 papers published under the MESH heading of 'Mesenchymal Stem Cells', the same search ran for the year 2007 reveals more than 4000 entries.

The use of autologous adult bone marrow derived stem cells in research can again be segregated into three main areas: nucleated cell isolates, isolated MSC's without culture expansion, and isolated MSC's with culture expansion. The most commonly used source of MSC's is bone marrow aspirate. Most of the adult bone marrow consists of blood cells in various stages of differentiation [29]. These marrow components can be divided into plasma, red blood cells, platelets, and nucleated cells (Fig. 1). The adult stem cell fraction is present in the nucleated cells of the marrow. Most of these cells are CD34+ heme



**Figure 1** Example of commercially available bedside computerized centrifuge which can concentrate platelets and bone marrow. While this approach is convenient for the practitioner, the downside is that the number of adult stem cells that can be concentrated is very low.



**Figure 2** Microscopy of mesenchymal stem cells in culture that have been allowed to produce their own extracellular matrix (dark area). This type of cell growth is called a tissue engineered construct (TEC). The cells can be seen migrating from this TEC to look for less crowded growth conditions.

progenitors (destined to differentiate into blood components), while very few are actually MSC's capable of differentiating into bone, cartilage, or muscle. In small animal models these CD34+ heme stem cells can transdifferentiate into MSC's, but in primates this does not appear to be the case [30]. As a result, that leaves the very small number of MSC's in the marrow as cells capable of differentiating into tissues of interest to pain medicine. Of note, this may be one of the reasons that commercially available centrifuge systems that concentrate marrow nucleated cells have not shown as much promise in animal research for cartilage repair as approaches where MSC's are expanded in culture to greater numbers.

Marrow nucleated cells are used every day in regenerative orthopedics. The knee micro fracture technique popularized by Steadman relies on the release of these cells into a cartilage lesion to initiate fibrocartilage repair in osteochondral defects [31]. In addition, this cell population has also been shown to assist in the repair of non-union fractures [32]. For this application, bed side centrifugation is commonly used. Again, these techniques produce a very dilute MSC population, usually a yield of one in 10,000–1,000,000 of the nucleated cells [33]. Despite this low number of MSC's, isolated bone marrow nucleated cells implanted into degenerated human peripheral joints have shown some promise for joint repair [34]. Our own research in this area showed some capability of regenerating bony cortex over a hip subchondral cyst, but in our experience, reliable cartilage repair in osteoarthritis seemed beyond the capability of this cell population.

Since it is MSC's that are capable of differentiating into cartilage, their use as cellular building blocks that can be implanted via fluoroscopy guided percutaneous procedures has some face validity. As discussed above, the number of MSC's that can be isolated from bone marrow is fairly limited. As a result, most research in cartilage regeneration has focused on the use of culture expanded cells [35–38]. Culture expansion is the practice of isolating cells and placing them in ex-vivo culture with a basal medium of nutrients and various growth factors (Fig. 2). The goal is usually to 'expand' cell numbers by 100–10,000-fold over several weeks. The most common method of isolating the very scarce MSC's from the much more numerous bone marrow nucleated cells is through adhesion to plastic in tissue culture [39]. MSC's will adhere to plastic, whereas the remainder of the nucleated cell population will not adhere. As a result, the adherent colonies are collected and then over several steps further 'filtered' out of the larger nucleated cell population using adhesion. Once a pure MSC population is isolated, the tissue culture is transferred to monolayer, where the cells can continue to grow adherent to the plastic flask, while they are covered by a thin layer of basal nutrient media and growth factors. As the cells cover the surfaces of the plastic flasks, they are periodically removed from those surfaces with trypsin and reseeded in a greater number of flasks with new media (nutrients and growth factors). This one cycle of cell collection, seeding, and feeding is known in the cell culture vernacular as a 'passage'. MSC's are usually harvested by the 3–5th passage (several weeks total culture time) and at that point have been grown to between 100–10,000 times more cells than harvested [40]. Longer term culture beyond 10 passages is thought to risk possible mutation in the cell line.

As discussed above, once cells are ready for reimplantation, they are usually transferred with growth factors to allow for continued cell growth and engraftment to the damaged tissue. At some point, a signal is introduced (either in culture or after transplant to the damaged tissue) for the cells to differentiate into the end tissue (in this discussion, cartilage). Various elements of the local microenvironment can effect MSC differentiation [1,18,32,41,42]. Several authors have shown that nano doses dexamethasone acts as a potent differentiating agent for MSC's toward a chondrogenic lineage [35,43,44]. It is very important to separate this nanogram range dose of corticosteroids from the approximately 1, 1000, 000 times more (milligram doses) used clinically. These larger doses (mg dose) have been shown to cause cellular apoptosis and joint destruction over time [45–49].

In addition, it is thought that a scaffolding material might be needed to allow the MSC's to attach and engraft [38–41]. Many different types of scaffolding material have been used. For example, MSC's can be seeded on an allograft such as an ACL ligament [50]. In addition, injectable, self assembling scaffolds are common such as fibrin glue, hyaluronans, or mixtures of both [51,52].

## Growth factors in regenerative medicine

Recombinant human growth factors have just begun to be used in modern clinical practice. These are proteins in the cytokine family that have the ability to promote growth of certain tissues. The first of these to be available in spinal surgery practices is the bone morphogenic protein (BMP) family. Both BMP-2 and BMP-7 are currently being used to promote fusion [53,54]. Animal trials with these growth factors may show some promise in repair of peripheral joints [55]. In addition, FGF-2 has also recently been used in unpublished, small animal studies to promote disc repair. The TGF family has also shown promise in the same areas [56].

The present case study reports on a successful clinical result using percutaneously implanted, autologous culture expanded MSC's with nucleated cells and physiologic doses of dexamethasone as a differentiating agent. This patient is treated with a completely autologous processes, where mesenchymal stem cells were isolated from the iliac crest via a fluoroscopy guided percutaneous procedure, ex-vivo culture expanded in a clinical lab set up for this purpose, and then reintroduced via fluoroscopy with autologous growth factors isolated from platelets as well marrow nucleated cells.

## Methods

The research protocol was approved through a non-profit Institutional Review Board (The Spinal Injury Foundation, Westminster CO). Inclusion criteria were as follows:

1. Male or female patients, 18–65 years of age.
2. MRI evidence of degenerative knee osteoarthritis.
3. Persisting intrusive pain resulting from the condition identified in (2). To ensure that the diagnosis of intrusive osteoarthritis was accurate, diagnostic blocks were used. Confirmation that a joint was a primary cause of the patients' pain was accomplished with a fluoroscopically guided injection of 0.75% Marcaine and 4% Lidocaine into the joint space followed by complete pain relief.

4. The patient had been evaluated by a board certified orthopedic surgeon and informed that they were a knee arthroplasty candidate.

### Exclusion criteria

1. Active inflammatory or connective tissue disease (i.e., lupus, fibromyalgia, RA).
2. Active non-corrected endocrine disorder potentially associated with symptoms (i.e. hypothyroidism, diabetes).
3. Active neurologic disorder potentially associated with symptoms (i.e., peripheral neuropathy, multiple sclerosis).
4. Severe cardiac disease.
5. Pulmonary disease requiring medication usage.
6. A history of dyspnea or other reactions to transfusion of homologous blood products.

### Pre-procedure data collection

1. CBC and SMAC to rule out unknown medical condition (within 3 months of procedure).
2. The patient was surveyed for activity level.

A 36 year-old male was actively recruited by his pain physician from a private interventional pain management practice based on his diagnosis of OA causing significant ongoing pain and disability, and their willingness to proceed with the study. This patient was provided with extensive informed consent prior to being enrolled in the study.

### Patient history

The patient is a 36 year-old male who presented with right greater than left knee pain that developed approximately 15 years prior through overuse and several injuries. Pain was daily, constant, increased with flexion, and between 5 and 9/10 in intensity. He had no prior advanced imaging or arthroscopic interventions.

On exam of his right knee, he had medial joint line tenderness with no effusion, instability, or limitation in range of motion.

A pre-treatment 3 T MRI showed: degenerative thinning of the medial femoral chondral surface and degenerative changes in both horns of the medial meniscus.

For one week prior to the marrow harvest procedure the patient was restricted from taking corticosteroids or NSAIDs. Coincident with this harvest procedure, approximately 200 cc of heparinized IV venous blood was drawn to be used for platelet

lysate (PL). To prepare platelet lysate, platelet rich plasma was prepared via centrifugation at 200g to separate plasma/platelets from the red blood cells. The supernatant was then drawn off and platelets pelleted at 100g centrifugation. The platelets were then re-suspended to a 500% concentration (approximately  $1 \times 10^9$  per ml). This was considered 100% platelet lysate, which was then diluted to between 10–20% as supplement for serum free cell culture media.

The 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 10 cc from the left PSIS area (cell yields upon initial marrow harvest and processing are discussed in [Table 1](#)).

Whole marrow was centrifuged at 100g for 4–6 min to separate the plasma from the RBCs. The plasma was removed, placed in a separate tube, and centrifuged at 1000g for 10 min to pellet the nucleated cell fraction. The nucleated cells were washed once in PBS, counted, and then re-suspended in DMEM + 10% PL and seeded at  $1 \times 10^6$  cells/cm<sup>2</sup> in monolayer flask culture. Cultures were incubated at 37 °C/5% CO<sub>2</sub> in a humidified environment. The culture medium was changed after three 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 s such that only the colony-forming MSCs detached. The MSCs were reseeded at a density of 12,000 cells/cm<sup>2</sup> in  $\alpha$ MEM + 5%, 10%, or 20% PL. Each culture was passaged 1:3 after reaching 40–50% confluence ([Table 2](#) shows MSC growth per day in monolayer culture and [Table 3](#) shows growth per passage. A passage was defined as a change of tissue culture medium).

After MSC's had been grown to the third passage, they were suspended in phosphate buffered saline (PBS). The patient returned to the clinic and was consented in writing. The patient was then placed prone on a fluoroscopy table and the bilateral PSIS area was prepped with betadine and sterile drapes. The skin and deeper tissues were then anesthetized with 1% Lidocaine. A sterile trocar was then inserted under fluoroscopy guidance to the superior medial corner of the iliac wing and 50 cc of marrow was drawn. This sample was sent to the lab for nucleated cell isolation. Red blood cells were separated from the majority of nucleated cells in the whole marrow via centrifugation at 200g for 5 min.

The patient returned to the OR a few hours later and was placed supine with the right knee bent at

**Table 1** Cell yields from marrow draw

Platelet count from whole blood (billion/ml)	Marrow draw site	Nucleated cell count (millions)	RBC count (billions)
1.09	Left PSIS	27	0.33
	Right PSIS	72	0.46

Note the disparity in the nucleated cell yield from left to right sites. Since MSC's represent approximately 1 in 10,000 nucleated marrow cells, nucleated cell yield is a likely proxy for total MSC's obtained by the marrow draw.

**Table 2** MSC growth per day in millions

Cell growth	
Day 0	1.86
Day 2	5.84
Day 3	
Day 4	
Day 5	21.4
Day 6	
Day 7	45.6
Day 8	
Day 9	
Day 10	
Day 11	
Days in colony = 8	

**Table 3** MSC growth in millions per tissue culture passage

Cell growth per passage	
P 0	1.86
P 1	5.84
P 2	21.4
P 3	45.6
P 4	
P 5	

45° and re-prepped using betadine and sterile gloves. A 25 gauge 2 in. needle was then inserted through a medial inferior approach under c-arm guidance. Once the joint space was attained, 0.25 cc of Isovue contrast diluted 50% with PBS was injected. Once good medial intra-articular flow was established, 2 cc hyaluronate sodium (Hyalgan) was injected, followed by 10 cc of fresh whole marrow and 45.6 million MSCs suspended in PBS. The patient was instructed to remain still for 1 h to allow for cell attachment and then was instructed to maintain activity as tolerated. He was also given a pulsed ultrasound device to be worn over the medial aspect of his right knee, 20 min a day for three weeks. The patient returned for

two additional 10% intra-articular knee platelet lysate injections (1 cc) at week 1 and week 2 (post-transplantation). With the two week post transplant platelet lysate supplementation, 1 ml of 10 ng/ml dexamethasone was also injected.



**Figure 3** Sample of 3 tesla MRI images used in the tracing and calculation of meniscus volumes pre- and post- MSC injection.

**Table 4** Cartilage Volume Analysis in mm<sup>3</sup>

Image	Area of measurement	Volume (n = 3)	STDEV	SE	% Change from pre-injection
Pre-injection	Cartilage surface	4668	329.28	190.34	
	Meniscus	4800	454.89	262.94	
One month	Cartilage surface	4659	319.01	184.4	-0.19
	Meniscus	5910	90.35	52.23	23.13
Three months	Cartilage surface	4228	151.48	87.56	-9.43
	Meniscus	5942	64.65	37.37	23.79

The cartilage volume analysis demonstrates an increase in meniscus volume beyond the measured standard deviation of serial measurements. The decrease in femoral cartilage surface volume is less than the measurement error of approximately 10% and is therefore not significant.

Modified VAS questionnaires and functional rating index [43–44] questionnaires were provided to the patient and administered before the procedure, one month after the procedure, and three months after the procedure. In particular, additional VAS data was calculated by multiplying the modified numerical VAS for knee pain by the frequency of that complaint. Range of motion measurements of the knee were measured by a physical therapist before the procedure, one month post-procedure and three 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 one month and at three months were obtained using matching 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 (see Fig. 3 and Table 4).

## Results

The pre and post-procedure MRI analysis demonstrated an increase in meniscus volume that was more than the standard deviation of the serial three ROI measurements. At three month follow-up, modified VAS scores decreased from 3.33 to 0.13.

## Conclusion

This case report shows MRI evidence of increased meniscus volume. While there has been evidence from animal models of cartilage regeneration using MSC's, this is the first case report of an increase in meniscus size in a human subject (of which we are aware). While the patient reported clinical response could have been due to the dexamethasone injection provided post transplant procedure, the levels injected (10 ng/ml) were approximately one million times lower than those used clinically (milligram or  $1 \times 10^{-3}$  vs. nanogram or  $1 \times 10^{-9}$ ). Diurnal variation in cartilage volume may explain these results as random variation, however, we are not aware of any studies that demonstrate large swings in cartilage volume based on loading.

It should be noted that without biopsy, there is no way to determine if the change was fibrocartilage or true hyaline cartilage. Of interest, this was a 'needle out/needle in' procedure with no activity restriction. Longer term follow-up of this patient will continue. Obviously, the generalizability of this technique to the larger population of patients with symptomatic osteoarthritis and traumatic knee injury is unknown.

As discussed in the above text, regenerative and cellular techniques likely hold great promise for expanding interventional pain management into the management of many disease states. The ability place medication in many areas of the body via X-ray guidance is a skill already possessed by interventional physicians. In addition, the clinical procedures used to extract cells via fluoroscopy involved placing a sterile trocar into key bony landmarks easily visible via fluoroscopy. Finally, while this laboratory based adult stem cell isolation and expansion was carried out using specialized person-

nel, the actual clinical lab was part of an interventional pain management practice.

## References

- [1] Cassiede P et al. Osteochondrogenic potential of marrow mesenchymal progenitor cells exposed to TGF-beta 1 or PDGF-BB as assayed in vivo and in vitro. *J Bone Miner Res* 1996;11(9):1264–73.
- [2] Chen YJ et al. Recruitment of mesenchymal stem cells and expression of TGF-beta 1 and VEGF in the early stage of shock wave-promoted bone regeneration of segmental defect in rats. *J Orthop Res* 2004;22(3):526–34.
- [3] Doucet C et al. Platelet lysates promote mesenchymal stem cell expansion: a safety substitute for animal serum in cell-based therapy applications. *J Cell Physiol* 2005;205(2):228–36.
- [4] Gordon EM et al. Capture and expansion of bone marrow-derived mesenchymal progenitor cells with a transforming growth factor-beta1-von Willebrand's factor fusion protein for retrovirus-mediated delivery of coagulation factor IX. *Hum Gene Ther* 1997;8(11):1385–94.
- [5] Reddi AH, Cunningham NS. Bone induction by osteogenin and bone morphogenetic proteins. *Biomaterials* 1990;11:33–4.
- [6] Frechette JP, Martineau I, Gagnon G. Platelet-rich plasmas: growth factor content and roles in wound healing. *J Dent Res* 2005;84(5):434–9.
- [7] Martineau I, Lacoste E, Gagnon G. Effects of calcium and thrombin on growth factor release from platelet concentrates: kinetics and regulation of endothelial cell proliferation. *Biomaterials* 2004;25(18):4489–502.
- [8] Anitua E et al. Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemostasis* 2004;91(1):4–15.
- [9] Bernardo ME et al. Optimization of in vitro expansion of human multipotent mesenchymal stromal cells for cell-therapy approaches: further insights in the search for a fetal calf serum substitute. *J Cell Physiol* 2007;211(1):121–30.
- [10] Muller I et al. Animal serum-free culture conditions for isolation and expansion of multipotent mesenchymal stromal cells from human BM. *Cytotherapy* 2006;8(5):437–44.
- [11] Schallmoser K et al. Human platelet lysate can replace fetal bovine serum for clinical-scale expansion of functional mesenchymal stromal cells. *Transfusion* 2007;47(8):1436–46.
- [12] Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. *Am J Sport Med* 2006;34(11):1774–8.
- [13] Aspenberg P, Virchenko O. Platelet concentrate injection improves Achilles tendon repair in rats. *Acta Orthop Scand* 2004;75(1):93–9.
- [14] Szilvassy SJ. The biology of hematopoietic stem cells. *Arch Med Res* 2003;34(6):446–60.
- [15] Barry FP. Mesenchymal stem cell therapy in joint disease. *Novartis Found Symp*, 2003. 249: 86–96; discussion 96–102, 170–4, 239–41.
- [16] Buckwalter JA, Mankin HJ. Articular cartilage: degeneration and osteoarthritis, repair, regeneration, and transplantation. *Instr Course Lect* 1998;47:487–504.
- [17] Caplan AI. Mesenchymal stem cells. *J Orthop Res* 1991;9(5):641–50.
- [18] Carter DR et al. Mechanobiology of skeletal regeneration. *Clin Orthop Relat Res* 1998;355(Suppl. 1):S41–55.
- [19] Johnstone B, Yoo JU. Autologous mesenchymal progenitor cells in articular cartilage repair. *Clin Orthop Relat Res* 1999;367(Suppl. 1):S156–62.
- [20] Luyten FP. Mesenchymal stem cells in osteoarthritis. *Curr Opin Rheumatol* 2004;16(5):599–603.
- [21] Magne D et al. Mesenchymal stem cell therapy to rebuild cartilage. *Trends Mol Med* 2005.
- [22] Murphy JM et al. Stem cell therapy in a caprine model of osteoarthritis. *Arthritis Rheum* 2003;48(12):3464–74.
- [23] Nevo Z et al. The manipulated mesenchymal stem cells in regenerated skeletal tissues. *Cell Transplant* 1998;7(1):63–70.
- [24] Noel D, Djouad F, Jorgense C. Regenerative medicine through mesenchymal stem cells for bone and cartilage repair. *Curr Opin Invest Drugs* 2002;3(7):1000–4.
- [25] Redman SN, Oldfield SF, Archer CW. Current strategies for articular cartilage repair. *Eur Cell Mater* 2005;9:23–32. discussion 23–32.
- [26] Tallheden T et al. Phenotypic plasticity of human articular chondrocytes. *J Bone Joint Surg Am* 2003;85-A(Suppl. 2):93–100.
- [27] Wakitani S et al. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. *J Bone Joint Surg Am* 1994;76(4):579–92.
- [28] Walsh CJ et al. Meniscus regeneration in a rabbit partial meniscectomy model. *Tissue Eng* 1999;5(4):327–37.
- [29] Verfaillie C, Blakolmer K, McGlave P. Purified primitive human hematopoietic progenitor cells with long-term in vitro repopulating capacity adhere selectively to irradiated bone marrow stroma. *J Exp Med* 1990;172(2):509–12.
- [30] Keyser KA, Morris JC, Kiem HP. Genetically modified CD34+ cells do not contribute to the mesenchymal compartment after autologous transplantation in the baboon. *Cytotherapy* 2005;7(4):345–52.
- [31] Steadman JR et al. An arthroscopic treatment regimen for osteoarthritis of the knee. *Arthroscopy* 2007;23(9):948–55.
- [32] Bruder SP, Fink DJ, Caplan AI. Mesenchymal stem cells in bone development, bone repair, and skeletal regeneration therapy. *J Cell Biochem* 1994;56(3):283–94.
- [33] D'Ippolito G et al. Age-related osteogenic potential of mesenchymal stromal stem cells from human vertebral bone marrow. *J Bone Miner Res* 1999;14(7):1115–22.
- [34] Centeno CJ et al. Partial regeneration of the human hip via autologous bone marrow nucleated cell transfer: a case study. *Pain Physician* 2006;9(3):253–6.
- [35] Bosnakovski D et al. Chondrogenic differentiation of bovine bone marrow mesenchymal stem cells (MSCs) in different hydrogels: influence of collagen type II extracellular matrix on MSC chondrogenesis. *Biotechnol Bioeng* 2006;93(6):152–63.
- [36] Gao J, Caplan AI. Mesenchymal stem cells and tissue engineering for orthopaedic surgery. *Chir Org Mov* 2003;88(3):305–16.
- [37] Guo X et al. Repair of large articular cartilage defects with implants of autologous mesenchymal stem cells seeded into beta-tricalcium phosphate in a sheep model. *Tissue Eng* 2004;10(11–12):1818–29.
- [38] Xiang Y et al. Ex vivo expansion and pluripotential differentiation of cryopreserved human bone marrow mesenchymal stem cells. *J Zhejiang Univ Sci B* 2007;8(2):136–46.
- [39] Pereira RF et al. Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice. *Proc Natl Acad Sci USA* 1995;92(11):4857–61.

- [40] Crisostomo PR et al. High passage number of stem cells adversely affects stem cell activation and myocardial protection. *Shock* 2006;26(6):575–80.
- [41] Cui JH et al. Effects of low-intensity ultrasound on chondrogenic differentiation of mesenchymal stem cells embedded in polyglycolic acid: an in vivo study. *Tissue Eng* 2006;12(1):75–82.
- [42] Risbud MV et al. Differentiation of mesenchymal stem cells towards a nucleus pulposus-like phenotype in vitro: implications for cell-based transplantation therapy. *Spine* 2004;29(23):2627–32.
- [43] Scutt A, Bertram P. Basic fibroblast growth factor in the presence of dexamethasone stimulates colony formation, expansion, and osteoblastic differentiation by rat bone marrow stromal cells. *Calcified Tissue Int* 1999;64(1):69–77.
- [44] Zhang Y et al. In vitro chondrogenic phenotype differentiation of bone marrow-derived mesenchymal stem cells. *J Huazhong Univ Sci Technol Med Sci* 2004;24(3):275–8.
- [45] Chrysis D, Ritzen EM, Savendahl L. Growth retardation induced by dexamethasone is associated with increased apoptosis of the growth plate chondrocytes. *J Endocrinol* 2003;176(3):331–7.
- [46] Chrysis D et al. Dexamethasone induces apoptosis in proliferative chondrocytes through activation of caspases and suppression of the Akt-phosphatidylinositol 3'-kinase signaling pathway. *Endocrinology* 2005;146(3):1391–7.
- [47] D'Lima DD et al. Prevention of chondrocyte apoptosis. *J Bone Joint Surg Am* 2001;83-A(Suppl. 2(Pt 1)):25–6.
- [48] Jones LC, Hungerford DS. The pathogenesis of osteonecrosis. *Instr Course Lect* 2007;56:179–96.
- [49] Nakazawa F et al. Corticosteroid treatment induces chondrocyte apoptosis in an experimental arthritis model and in chondrocyte cultures. *Clin Exp Rheumatol* 2002;20(6):773–81.
- [50] Li F, Jia H, Yu C. ACL reconstruction in a rabbit model using irradiated Achilles allograft seeded with mesenchymal stem cells or PDGF-B gene-transfected mesenchymal stem cells. *Knee Surg Sports Traumatol Arthrosc* 2007;15(10):1219–27.
- [51] Silverman RP et al. Injectable tissue-engineered cartilage using a fibrin glue polymer. *Plast Reconstr Surg* 1999;103(7):1809–18.
- [52] Radice M et al. Hyaluronan-based biopolymers as delivery vehicles for bone-marrow-derived mesenchymal progenitors. *J Biomed Mater Res* 2000;50(2):101–9.
- [53] Hamilton DK et al. Outcomes of bone morphogenetic protein-2 in mature adults: posterolateral non-instrument-assisted lumbar decompression and fusion. *Surg Neurol* 2008.
- [54] White AP et al. Clinical applications of BMP-7/OP-1 in fractures, nonunions and spinal fusion. *Int Orthop* 2007;31(6):735–41.
- [55] Ishii I et al. Healing of full-thickness defects of the articular cartilage in rabbits using fibroblast growth factor-2 and a fibrin sealant. *J Bone Joint Surg Br* 2007;89(5):693–700.
- [56] Blaney Davidson EN, van der Kraan PM, van den Berg WB. TGF-beta and osteoarthritis. *Osteoarthritis Cartilage* 2007;15(6):597–604.

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