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Stem Cell Therapy for the Treatment of Hip Osteonecrosis: A 30-Year Review of Progress

Philippe Hernigou, MD, Matthieu Trousselier, MD, François Roubineau, MD, Charlie Bouthors, MD, Nathalie Chevallier, MD*, Helene Rouard, MD*, Charles Henri Flouzat-Lachaniette, MD

*Department of Orthopaedic Surgery, University Paris East (UPEC), Hôpital Henri Mondor, Creteil, *EFS Cell Therapy Facility, University Paris East (UPEC), Hôpital Henri Mondor, Creteil, France*

Avascular necrosis of the femoral head is caused by a multitude of etiologic factors and is associated with collapse with a risk of hip arthroplasty in younger populations. A focus on early disease management with the use of stem cells was proposed as early as 1985 by the senior author (PH). We undertook a systematic review of the medical literature to examine the progress in cell therapy during the last 30 years for the treatment of early stage osteonecrosis.

Keywords: *Hip, Osteonecrosis, Cell therapy, Mesenchymal stromal cells, Tissue engineering*

Osteonecrosis of the femoral head (ONFH) is a debilitating, painful, progressive, and refractory disease that has multiple etiologic risk factors. It is caused by bone cell death, which itself has various causes, leading to articular cartilage collapse and subsequent osteoarthritis. ONFH primarily influences patients aged from 30 to 40 years; in addition, bilateral hip joints are involved in 75% of patients. Causes include corticosteroids, alcohol abuse, previous trauma, hemoglobinopathy, Gaucher disease, coagulopathies, and other diseases. Disease progression leads to collapse of the affected femoral head and development of osteoarthritis. Outcomes of total hip arthroplasty (THA) for these young and active patients have some problems, primarily due to the limited lifetime and durability of THA. As a result, there has been an increased focus on early interventions for ONFH aimed at preservation of the native articulation. Core decompression is currently the most widely accepted treatment at the early stage of avascular osteonecrosis (AVN); however, due to limited efficacy, its use has been debated.

The rationale for the use of cytotherapy in osteonecrosis as well as techniques for implantation of osteogenic progenitor cells (autologous or allogenic) were evaluated, and began as a research program in 1985 by the senior author (PH). Bone marrow mesenchymal stem cells (BMMSCs) have powerful capabilities for self-proliferation and multi-potential differentiation, and can be induced to undergo osteogenesis. Elucidation of the characteristics of BMMSCs of a controlled culture *in vitro* and of osteogenic differentiation after implantation could lead to new breakthroughs for the treatment of ONFH. The first patient who received mesenchymal stem cells (MSCs) for ONFH had surgery in 1989 at Henri Mondor Hospital. In 1993, Hernigou and Beaujean[1,2] first described a technique for injecting mesenchymal stem cells combined with standard core decompression to introduce biologics into an area of necrosis. The first mid-term results were reported in 2002.[3] In a study of 189 hips (116 patients), mesenchymal stem cells (in the form of concentrated iliac crest bone marrow) were injected through a core decom-pression tract into the area of necrosis. Patients with early (precollapse) disease had excellent results at 5 to 10 years of clinical follow-up, with only nine of 145 hips requiring THA.

The aim of this paper is to present the following: the rationale for the use of autologous bone marrow concentrate grafting in hip osteonecrosis; the technique for treating hip osteonecrosis with MSCs obtained from autologous concentrated bone marrow; the possibility of using *ex vivo* expanded autologous bone marrow-derived

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Correspondence to: Philippe Hernigou, MD
Department of Orthopaedic Surgery, University Paris East (UPEC), Hôpital Henri Mondor, Creteil 94010, France
Tel: +33-1-49-81-26-01, Fax: +33-1-49-81-26-08
E-mail: philippe.hernigou@wanadoo.fr

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stem cells; different techniques of MSC administration in the hip; the results and mechanism of healing of the hip osteonecrosis with progenitor cell treatment; the number of cells that are necessary for femoral head repair; and the safety of cytotherapy in the treatment of hip osteonecroses.

RATIONALE FOR STEM CELL THERAPY IN HIP OSTEONECROSIS

The relationships between bone marrow and osteonecrosis of the proximal femur are complex.[4] Changes in the bone marrow signal are observed on magnetic resonance imaging (MRI) scans of patients with osteonecrosis. There is an increase in the amount of fatty marrow in the intertrochanteric portion of osteonecrotic hips. Abnormalities of osteogenic stem cells are present in the bone marrow of some of these patients. Steroids have been shown to produce adipogenesis and stimulate fat-specific genes in cloned bone-marrow cells; following corticosteroid therapy in osteonecrotic patients, abnormalities have been demonstrated in the bone marrow of the iliac crest, with a decrease in the stem cell pool.[5] The effects of steroids on cloned bone marrow cells *in vitro* include the production of adipogenesis and the stimulation of fat-specific genes.

As a consequence, intramedullary vascularity is altered and this may be a predisposing factor for osteonecrosis since changes in bone marrow and bone remodeling are linked. Another consequence is the lack of osteogenic cells, which could influence two different events in the pathogenesis of osteonecrosis; the occurrence of osteonecrosis itself and the bone repair occurring after osteonecrosis. A decrease in osteogenic stem cells in the femoral head has been observed beneath the sequestrum and in the intertrochanteric region. This has also been confirmed by the observation of the extent of osteocyte death in the proximal femur seen in patients who underwent total hip replacement (THR) for osteonecrosis. One of the reasons for insufficient creeping substitution in bone remodeling after osteonecrosis in the femoral head may be the small number of progenitor cells present in the femoral head in these patients. Although both research and clinical studies have shown that dead bone may be replaced by living bone, the osteogenic potential for repair is low in osteonecrosis. Bone marrow activity[5] in the proximal femur of patients with corticosteroid-induced ONFH was evaluated and compared with a control group without ONFH. A decrease in the number of MSCs was found outside the area of ONFH in patients with a corticosteroid induction. Reconstruction and repair has been observed after core decompression, but is usually incomplete. Grafting with autologous bone marrow could lead to increased repair in osteonecrosis of the hip.

Autologous bone marrow transplantation was therefore proposed for the treatment of osteonecrosis. The effectiveness of bone marrow mononuclear cells may be related to the availability of stem cells endowed with osteogenic properties arising from an increase in the supply of such cells to the femoral head through bone marrow implantation.[6-10] Another possible explanation for the therapeutic effect of bone marrow implantation is that injected marrow stromal cells secrete angiogenic cytokines, resulting in increased angiogenesis and subsequent improvement in osteogenesis. Finally, bone marrow-derived mononuclear cells are able to elicit the formation of new blood vessels by the presence of endothelial cell progenitors or hemangioblasts in this cell fraction. This may be due both to the supply of progenitor cells and to angiogenic cytokines produced by bone marrow cells. Endothelial progenitors can actively engage in vasculogenesis in tissue devoid of vessels, and in neoangiogenesis from the pre-existing capillaries. Besides the generation of new capillaries, the growing endothelia enhance mobilization and growth of mesenchymal progenitors through the angiopoietin 1-Tie2 pathway, which generates pericytes and vascular mural cells required for new vessel growth and stabilization. A broad capacity of differentiation of perivascular mesenchymal cells has been shown, and participation of perivascular mesenchymal progenitors in repair of adjacent tissues has been described both in experimental models and humans. In addition, local ischemia that activates HIF1A signaling and mobilization of circulating progenitors through the SDF1-dependent pathway, may supply permanent stimuli for blood vessel repair and a supply of new cells for bone regeneration.

Adult MSCs represent usually a heterogeneous population of cells, with a positive immunophenotype for STRO-1, CD73, CD146, and CD106 and a negative one for CD11b, CD45, CD34, CD31, and CD117.[6-10] MSCs act *via* multifaceted pathways not yet completely understood in order to augment regeneration, and includes mechanisms that mediate homing of administered MSCs to sites of injury. However, two main inherent functions of MSCs can be distinguished. The first is the secretory or "trophic" function of MSCs, which includes the secretion of a wide spectrum of factors with immunomodulatory, anti-inflammatory, antiapoptotic, proangiogenic, proliferative, or chemoattractive capacities, among others. Second, administered MSCs are thought to orchestrate the differentiation process together with differentiated or undifferentiated resident cells for functional tissue restoration.

Given the different modes of MSC delivery as well as the time and efforts necessary for performing meaningful randomized controlled clinical trials, the search for an optimized procedure has remained elusive. However, several studies have been conducted that use mesenchymal stromal cell-based approaches in the clinical setting, and some of the basic principles of stromal cell delivery are now outlined. A successful regenerative approach based on the use of autologous material usually comprises the harvest of cells either derived from bone marrow by, for example, aspiration from the iliac crest or by surgical removal from a donor site.

TECHNIQUE FOR TREATMENT OF HIP OSTEONECROSIS WITH MESENCHYMAL STEM CELLS

Hernigou and colleagues[11,12] pioneered the clinical application of a cell-based strategy for the treatment of AVN, percutaneously injecting an autologous bone marrow concentrate into the necrotic area of femoral heads. The strategy is driven by the hypothesis that injected bone marrow cells can repopulate the trabecular bone structure and subsequently revitalize and remodel the necrotic bone. For this technique, a bone marrow aspirate (BMA) is harvested from the iliac crest and the mononuclear cell fraction is isolated. After core decompression, the cell suspension is injected into the necrotic area.

Bone Marrow Aspiration and Cell Harvesting

Bone marrow can be collected from either the anterior or posterior part of the iliac crest. For a supine patient the target will be the anterior part of the iliac crest; for a patient in a lateral position then the target will be either anterior or posterior; and if the patient is face down then the posterior part of the iliac crest will be the target. For patients of normal weight, a direct puncture of the needle is appropriate, but obese patients require a 2-mm incision to be made at the site of collection. Collection of bone marrow from the iliac crest is accomplished by the use of a single beveled aspirating needle. A standard 10-mL syringe should be used to obtain the BMA. Preparation of the needle and syringe prior to insertion involves the rinsing of the needle and aspiration syringe with a heparin solution. The needle is turned 45° during successive aspirations to reorient the bevel, thereby affording aspiration from the largest possible space. After one full turn, the needle is moved towards the surface through the same insertion site and successive aspirations are again begun, always turning the needle 45° after each aspiration. The aspirated marrow is richer in stem cells when it is aspirated in small fractions,[13] reducing the degree of dilution by peripheral blood. All aspirates are pooled in plastic bags containing cell culture medium and anticoagulant solution. Pooled aspirates are filtered to separate cellular aggregates and fat, and the mononuclear fraction is separated by standard procedures for injection.

The bony anatomy of the human ilium was studied to help the surgeon perform bone marrow aspiration from the iliac crest in the thickest part of the ilium.[14,15] The minimum thickness of the spongious bone in an iliac wing (transverse thickness between the two tables) is an important factor in ensuring the safe placement of a trocar between the two tables of the iliac wing. For example, with an 8-gauge (3.26 mm) trocar, one can consider that if the transverse thickness of the spongious bone of the iliac wing is < 3 mm, it will be difficult to insert the trocar safely between the two tables. Iliac wing sectors were formed by extending lines from the anterior superior and posterior superior iliac spine through the center of the acetabulum and the bisecting line of the angle formed by the two previous lines, resulting in anterior and posterior halves. The mean length of the iliac crest is 24 cm, and each half of the iliac wing was divided into three sectors by drawing lines from points 4 cm apart on the iliac crest to the center of the hip. These lines, which were approximately perpendicular to the curve of the iliac crest, defined the six sectors. The corresponding sectors can be found and marked on the patient by using the same technique. A map was constructed indicating the thickness of the spongious bone in each sector. The thickness data was used to create a map that identifies the sites where bone marrow can be obtained with a trocar of 3-mm diameter according to the thickness of the spongious bone. Sectors 2, 3, and 6 appear to be more favorable for accommodating a 3-mm diameter trocar. Sectors 1, 4, and 5 comprise the areas with the thinnest parts of the iliac crest, with some areas being thinner than the trocar diameter. The sector system reliably predicted safe and unsafe areas for trocar placement. In cadavers, dissection demonstrated nine vascular or neurologic lesions created when trocars were introduced into sectors 1, 5, and 6.

Intraosseous Injection of Mesenchymal Stem Cells

Patients were placed on a table with two image intensifiers with a C-arm. The decompression was performed with a percutaneous approach using a 3-mm diameter trephine trocard (Mazabraud, Collin, France). The bone marrow was injected into the femoral head using a small (Mazabraud) trocar. The instrument was introduced through the greater trochanter, as in conventional core decompres-

sion. Its position in the femoral head and in the necrotic segment was monitored with biplane fluoroscopy. Since, at the time of treatment, the plain radiographs showed little if any evidence of necrosis, the preoperative MRI scans are used together with the image intensifier views to determine the site of the lesion. Before the injection of the bone marrow, a few milliliters of contrast can be injected, in order to check the area in the femoral head through which the injected bone marrow will spread. It has been established that the contrast medium did not damage the bone progenitor cells.

Although the bore of the trocar is small compared with the trephines normally used for core decompression, femoral head and trochanteric region pressure measurements have shown that even a small hole relieved the intraosseous pressure. During the bone marrow injection, the pressure in the femoral head was found to rise, but a normal pressure pattern was restored once the injection was finished, exactly as in intraosseous pressure measurements. In our patients, no complications were observed during anaesthesia; in particular, there was no reduction in oxygen saturation, and no change in pulse rate or blood pressure.

THE RESULTS AND MECHANISM OF REPAIR OF HIP OSTEONECROSIS WITH PROGENITOR CELLS

Hernigou and Beaujean[3] have published clinical data on their experience. They treated 189 hips in 116 patients with autologous BMCs and had a follow-up of 5 to 10 years. Satisfactory results were achieved in the majority of patients according to improvement of the Harris hip score, radiographic assessment and refusal of THA. The prognosis was not only highly related to the stage of disease, but also to the progenitor cells transplanted. When patients were operated on before collapse and received a greater number of BMC injections, a better outcome could be expected. In 2008, Hernigou et al.[16] retrospectively analyzed 534 hips in 342 patients with ONFH treated with autologous BMC transplantation. The results were really encouraging. They showed that the volume of necrosis would decrease from 26 cm^3 to 12 cm^3 in 371 patients with an average follow-up of 12 years. There were only 94 patients who progressed to THA. The author concluded that the best indication for cytotherapy of ONFH was in the precollapse stage when the hip was symptomatic; and in some patients with Steinberg stage III, ONFH successful outcome could be obtained in 5 to 10 years.

In another report from Gangji and colleagues,[17,18] patients with ONFH were treated by injection of bone marrow stromal cells (BMSCs). Osteoprogenitors and osteoblasts from bone marrow were separated and expanded *in vitro*, and injected into the necrotic zone after differentiation under autologous conditions. Pain reduction, necrotic lesion decrease and functional improvement were recorded in the early period, and only minor side effects were found.

Papakostidis et al.[19] and Hernigou et al.[20] investigated through literature review whether implantation of autologous BMA containing high concentrations of pluripotent mesenchymal stem cells into the core decompression track would improve the clinical and radiological results as compared with the classical method of core decompression alone. The primary outcomes of interest were structural failure (collapse) of the femoral head and conversion to THR. They identified 496 citations between 2002 and 2015, and selected papers of interest.[21-30] Their findings indicate that the application of autologous bone marrow concentrate (autologous cell therapy) in combination with core decompression in osteonecrotic femoral heads is superior to core decompression treatment, as it was found to markedly decelerate the progression of the disease to the stage of femoral head collapse, and also limit the need for THA. Some component studies also reported on clinical results, demonstrating that autologous cell therapy in addition to core decompression for the treatment of ONFH resulted in reduction of painful joint symptoms and improvement in the Harris hip score as compared with sole use of the core decompression technique.

Clinical outcomes are usually analyzed with hip score, collapse, and the need for THA. The reduction of volume[31,32] and restoration of original MRI signals of a living bone marrow is sometimes observed. However, it appears that there are many variations of the signal when abnormal tissue (normal tissue, ossification, fibrous tissue) is repaired with absence of collapse; furthermore, when scaffolds are used (hydroxyapatite), the presence of bone substitute remains visible in the femoral head for a long time (several years), and of course acts as an artifact to evaluate the exact repair. Therefore, at this moment, most of the clinical studies keep as an outcome the absence of collapse during the evolution.

HOW MANY CELLS ARE NECESSARY FOR TISSUE REPAIR?

The number of MSCs in a normal femoral head was evaluated by Hernigou et al.[5] and Homma et al.[33] by bone marrow aspiration and femoral head fragmentation. Bone mar-

row was collected by aspiration from the femoral head of patients receiving THA. The data showed that the total number of MSCs present in 1 cm^3 of a femoral head was on average of 700 ± 264 MSCs per cm^3. Since the femoral head has an average volume of 50 cm^3, a total of 35,000 MSCs may be considered as a useful approximation of the number of MSCs present in a femoral head. This number may be considered as the target number to load in an osteonecrotic femoral head to reestablish the same number of MSCs as in a normal femoral head. The influence of the number of progenitors on the results of treatment of hip osteonecroses of the femoral head was evaluated in patients treated through grafting with autologous bone marrow. MRI observation in a series of osteonecroses demonstrated that the volume repair was on average 15 cm^3 with an injection of a bone marrow graft of 20 mL containing approximately 2,500 progenitors per mL. In adult bone remodeling, these processes of bone formation take place in the context of the basic multicellular unit described by Parfitt et al.[34] and Frost.[35] Osteoblasts begin secreting matrix within a day, and matrix synthesis increases over several days to a maximum rate of approximately 1.5 μm per day over an area of approximately 150 $μm^2$ per osteoblast, resulting in synthesis of approximately 225 $μm^3$ per day per osteoblast. It is estimated that the volume of bone matrix made by one osteoblast is approximately 5,000 $μm^3$. The osteocyte density is reported to be 0.000047 osteocytes/$μm^3$ in cancellous bone. As a first approximation, based on a mean bone matrix of 33% in cancellous bone, one can estimate the number of osteocytes in 1 cm^3 of cancellous bone to be in the range of 20 million. There are approximately 20 million osteoblasts or osteocytes per mL of new bone. Since there are approximately 2,500 progenitors per mL of prepared marrow graft, each must have divided a minimum of 12 or 14 times to obtain 1 mL of new bone, assuming that all the bone marrow graft retained the ability to make trabecular bone ($2,500 \times 2^{14} = 20$ million osteoblasts). Histologic observations have demonstrated that the ratio of trabecular bone is 1/3 in the femoral head, the others 2/3 being fat and hematologic cells. Assuming that in the repair of 15 cm^3 in the femoral head that 1/3 is bone, this means that 5 cm^3 of bone were obtained.

CYTOTHERAPY OF HIP OSTEONECROSIS: CHALLENGES AND PROSPECTS

Important Variations of the Number of MSCs Are Observed in Patients and May Be a Limit of the Technique

Decreased MSCs have been described in patients with corticosteroid-induced and alcohol-induced hip osteonecroses. MSCs were shown to be abnormal in some hematological disorders and decreased in others conditions, as tobacco. The number varied also depending on the age of patient.

Tissue engineering is probably the solution[36,37] to achieving a regular number of cells in patients. It combines bone marrow cells or MSCs, synthetic scaffolds and molecular signals (growth or differentiating factors) in order to form hybrid constructs. In a classical approach, bone tissue engineering consists of harvesting bone marrow from a patient, isolating MSCs by their adherence to tissue culture plastic, expanding and differentiating those cells in culture to a sufficient number and then seeding them onto a suitable synthetic scaffold prior to implantation into the same patient.[38-41] The autologous approach to isolation and osteogenic differentiation of MSCs is however highly demanding in terms of logistics, production and safety of culture conditions, leading to a costly therapeutic procedure. The association of biomaterials and osteoprogenitor cells raises technical challenges (i.e., cell sources, types, doses, and timing) and regulatory issues (devices with medicinal drugs) for the implementation of clinical trials. Several methods could be used to increase the MSC population and its osteogenic differentiation in the pathological area. In a classical approach, bone tissue engineering consists of harvesting bone marrow from a patient, isolating MSCs by their adherence to tissue culture plastic, expanding and differentiating those cells in culture and then seeding on a suitable synthetic scaffold prior to implantation into the same patient. The autologous approach for isolation and osteogenic differentiation of MSCs is highly demanding in terms of logistics, production and safety of culture conditions leading to a costly therapeutic procedure. However, the number of MSCs after 3 weeks of tissue culture usually expands to about 5 million MSCs per mL. Thus, according to the reported culturing time that ranges from 10 days to 3 weeks, the number of cultured MSCs could range from 100,000 to 20 million cells. The authors of this paper have already begun such treatment in France. This work is supported by the 7th Framework Program of the European Commission through the REBORNE (Regenerating bone defects using new biomedical engineering approaches) project (Health-2009-1.4.2-241879).

Allogenic Bone Marrow-Derived Stem Cell Therapy

A unique advantage of MSCs is their potential for allogenic cell delivery in immunocompetent patients. Their immune-privileged characteristic is partially due to the lack of expression of major histocompatibility complex (MHC) II antigens that are responsible for immune rejection, al-

though MHC II expression could be induced by interferon gamma (IFN-γ) stimulation. In addition, MSCs lack the expression of co-stimulatory molecules that activate T cells, including CD40, CD80, and CD86. MSCs possess the immunomodulatory effects of T cell and B cell proliferation inhibition. Hernigou et al.[42] previously reported on the use of allogenic stem cells in osteonecrosis treatment. They reported the case of a patient who had osteonecrosis of the humeral head secondary to sickle-cell disease. Treatment with a bone marrow allograft led to a favorable outcome and total repair of the osteonecrosis after a follow-up of 4 years. The transplantation was performed in February 1992 after administration of a conditioning regimen of busulfan (16 mg per kg of body weight), cyclophosphamide (200 mg per kg of body weight), and total lymphoid irradiation in order to suppress the immune response and to eliminate hematopoietic precursors. The bone marrow donor was a human leukocyte antigen-identical sibling for whom a mixed-leukocyte culture was nonreactive; the donor was heterozygous for sickle-cell anemia. Such a treatment with expanded allogenic stem cells could decrease the price of the procedure.

Gene-Transfected BMMSCs Transplantation

The method of transferring target genes into BMMSCs *via* a carrier, and implanting them into osteonecrotic areas of the femoral head is an option for research. Bone morphogenetic proteins (BMPs) can facilitate bone formation and promote ectopic osteogenesis. Xiao et al.[43] showed that BMPs could repair experimental defects of ONFH using BMMSC-seeded bio-derived bone materials combined with rhBMP-2. Tang et al.[44] associated porous beta-tricalcium phosphate loaded with BMP-2-gene-transduced BMMSCs for the treatment of early-stage ONFH. Wen et al.[45] used transplantation of hepatocyte growth factor transgenic autologous BMMSCs to enhance blood vessel regeneration and bone reconstruction in an ONFH model.

CONCLUSION

In future research, several questions will need to be addressed. For example, is the differentiation potential of MSCs from different sources (bone marrow, fat, and periosteum) the same? Are their functional abilities after repeated culture the same? How different are bones formed by implanted MSCs from normal bones in terms of histology and biomechanics? In addition, the risk of forming cancer at the implanted site should be evaluated. Hernigou and colleagues[46,47] found that patients treated with cell therapy do not have a greater incidence of cancer than the rest of the population. They analyzed the occurrence of cancers by follow-up, cell number, sites of cancer, age, gender, and the pathology that was treated, and found that the risk of cancer was not increased in patients with longer follow-ups or in patients who had received a higher number of MSCs. However, this study was performed with autologous MSCs from bone marrow; there are no reports of implantation with allogenic MSCs or expanded MSCs with long-term follow-up.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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