Review

Osteonecrosis repair with bone marrow cell therapies: State of the clinical art

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Abstract

Introduction: Hip osteonecrosis is a pathological condition resulting from cellular impairment due to reduction in osteoblast activity and local mesenchymal stem cell populations. Cell-based therapies might aid in overcoming these deficiencies by providing stem cells and other progenitor cells to potentially improve the local cellular environment in the affected hip.

Methods: A PubMed search, using the search terms “hip osteonecrosis” and “mesenchymal stem cells”, was conducted in December 2013. A total of 15 publications were identified and reviewed for clinical outcomes.

Findings: Clinical studies of patients with osteonecrosis treated with mesenchymal stem cells showed beneficial effects. No unexpected adverse events were identified in these studies. Core decompression was the usual method for autologous bone marrow cell implantation into the femoral head. However, other methods have been used such as arterial or venous delivery. A rationale for the use of cytotherapy, as well as the different descriptions of the techniques of implantation MSCs (autologous vs. allogenic, concentration vs. expansion), is provided in the context of treating hip osteonecrosis. Current problems and future challenges with cytotherapy and associated techniques are discussed. This article is part of a Special Issue entitled “Stem Cells and Bones”.

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Introduction

Osteonecrosis of the femoral head (ONFH), except after trauma, is frequently associated with various risk factors, such as alcohol abuse, corticosteroid treatment, sickle cell disease or other hemoglobinopathies, coagulopathies, inflammatory or autoimmune diseases, organ transplantation, Gaucher’s disease, pregnancy, and other factors. Despite these many potential causes of ONFH, the pathophysiology remains uncertain. Abnormalities in the number or in the function of bone progenitor cells in osteonecrosis have been described [1,2]. The hypothesis that the disease had a cellular origin [3–5], or could be related to a mechanism that results in an imbalance between osteoblast formation and necrosis [6–8] was confirmed.

While the pathogenesis of osteonecrosis is still unclear, it can be viewed as a vascular and bone disease with altered bone remodeling. The combination of vascular and bone pathology contributes to the development of osteonecrosis, which leads to inadequate bone repair that advances to subchondral fracture. Based on the hypothesis that ONFH has a cellular origin, treatments incorporating cell-based therapy (“cytotherapy”) have great potential. However, the number of cells needed to stimulate osteonecrosis repair and the best way to deliver these cells are still unknown.

A PubMed search using the search terms “osteonecrosis”, and “cell therapies in human osteonecrosis” or “autologous bone marrow”, or “mesenchymal stem cells” was conducted in December 2013. About 15 articles were retrieved from the search and analyzed. No formal meta-analysis of the retrieved articles was carried out because of the heterogeneity of the populations, interventions, lengths of follow-up and variation in cell therapy treatments. Most of the clinical data in osteonecrosis are about bone marrow mesenchymal stem cells; therefore, those from adipose origin are excluded in our study. Core decompression (CD) has been widely used to delay progression of osteonecrotic lesions, which if left untreated end up destroying the femoral head. In the 1990s, injection of autologous bone marrow-derived cells into the femoral head during early-stage ONFH was proposed [9]. However, other methods for delivery of autologous cells have been used, including arterial or venous delivery.

A rationale for the use of cytotherapy, as well as the different descriptions of the technique of implantation of osteogenic progenitor cells (autologous or allogenic), is provided in the context of treating hip osteonecrosis. Current problems and future challenges with cytotherapy and associated techniques are discussed. The aim of this paper is to present: the rationale for use of autologous bone marrow concentrate grafting in hip osteonecrosis; the technique for treating hip osteonecrosis with mesenchymal stem cells (MSCs) obtained from autologous concentrated bone marrow; the possibility of using ex vivo expanded autologous bone marrow derived stem cells; different techniques of MSC administration in the hip; the results and mechanism of healing of the hip osteonecroses with progenitor cell therapy; the number of cells that are necessary for femoral head repair; and the safety of cytotherapy in the treatment of hip osteonecroses.

MSC levels present in a non-pathologic femoral head

The number of MSCs in a normal femoral head was evaluated by Hernigou [2] and Homma [10] by bone marrow aspiration and femoral head fragmentation. Bone marrow was collected by aspiration from the femoral head of patients receiving total hip arthroplasty. The needle was rinsed with a heparin solution, and introduced by hand through the femoral head to harvest bone marrow. Once in the femoral head, bone marrow was aspirated. The number of MSCs also was assessed by femoral head fragmentation. Fragments were generated from the femoral head by slicing the femoral head, yielding sections that were cut into cubes and pilled into particles. The femoral head pieces were immersed in a solution of cultured medium. After 3 days, cells were isolated from the cultures (non-adherent and adherent cells) and viable cells counted to evaluate the number of mononuclear cells and MSCs. There was a relationship between the number of MSCs obtained by bone marrow aspiration and the number of MSCs released by femoral head fragmentation. The data showed that the total number of MSCs present in 1 cm³ of a femoral head was on average of 700 ± 264 MSCs per cm³. Since the femoral head has an average volume of 50 cm³, 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.

Reduced MSC levels in patients with ONFH

Bone-marrow progenitor cell activity in the proximal femur of patients with corticosteroid-induced ONFH was evaluated and compared to a control group of patients without ONFH [2]. A decrease in the number of MSCs was found outside of the area of ONFH in patients with corticosteroid-induced ONFH. This reduction is in part related to the absence of MSCs in the osteonecrotic lesion itself, along with a global reduction in MSCs in the proximal part of the femur. For example, if an osteonecrotic lesion takes up a volume of 30% of the femoral head containing 36,000 MSCs, the estimated total number of MSCs in the femoral head will be reduced to 24,000 MSCs (36,000–12,000). However, if the MSC concentration outside the osteonecrotic zone also is lower, then the magnitude of loss is even greater. Consider a reduction of the MSC concentration outside the osteonecrotic zone to 200 MSCs per cm³ in the femoral head, as can be observed in patients with corticosteroid treatment, which would result in a femoral head containing just 3300 MSCs.

In addition to the number of MSCs present in the femoral head, there is also evidence that the MSCs present are not as active as MSCs in normal femoral heads. For example, Suh et al. [5] analyzed differentiation of MSCs from the proximal femur in 33 patients with alcohol-related ONFH. They observed that the MSCs showed a reduced potential to differentiate, and suggested that down-regulation of osteogenic potential could result in the onset of ONFH. A decrease in the level of osteogenic stem cells in the femoral head has also been observed beneath the sequestrum and in the intertrochanteric region [4]. In support of this finding, extensive osteocyte death was observed in the proximal femur for patients having total hip replacement for osteonecrosis [3]. An inadequate number of progenitor cells present within the femoral head may account for insufficient remodeling of new bone and osteonecrosis. The loss of osteogenic cells could influence two different events in the pathogenesis of osteonecrosis: the occurrence of osteonecrosis itself and the bone repair that occurs after osteonecrosis. For example, in patients with osteonecrosis following corticosteroid therapy, abnormalities have been demonstrated in the bone marrow of the iliac crest, including a decrease in the overall number of stem cells present [1]. Steroids also have been shown to induce adipogenesis and stimulate up-regulation of fat-specific genes of cloned bone-marrow cells in vitro cultures. All of which suggests that the MSCs would display a reduced osteogenic capability. Furthermore, it isn’t clear if initiation of osteonecrosis is due just to a decrease in MSC osteogenic potential, in the level of osteogenic cells, or is more likely, that these two factors, along with others contribute. However, it would be logical to hypothesize that a treatment in which osteogenic cells are introduced to the necrotic site directly would increase the level of progenitor cells, and thus improve bone remodeling by incremental substitution, thereby preserving the integrity of the femoral head.

Rationale for cell-based strategies in avascular osteonecrosis

To enhance tissue repair, autologous mesenchymal stem cells represent a highly promising candidate among several options for cell based therapeutic approaches. Adult mesenchymal stromal cells...
can be isolated from bone marrow [11,12]. These cells have the multi-
potential capacity for differentiation into osteoblasts [12]. Bone-
marrow derived mononuclear cells also promote formation of new
blood vessels due to the presence of endothelial cell progenitors or
hemangioblasts in the bone marrow concentrate [13]. Angiogenesis
may be promoted both by the increased supply of progenitor cells and
angiogenic cytokines produced by the bone marrow cells. MSCs also
can release a variety of growth factors to facilitate tissue regeneration
in the microenvironment [14]. VEGF-A is frequently produced by vascu-
lar and tumor cells, as well as MSCs. Release of VEGF from MSCs can pro-
mote recruitment of endothelial cells for angiogenesis in ischemic tissue
and endothelialization in injured arteries [11,12].

Thus, the multiple capabilities of MSCs, including their homing
ability to injury sites, their paracrine secretions enhancing cell migra-
tion, differentiation, and angiogenesis make them an ideal cell type to
mimic a bone autograft by demonstrating all of the key components
required for bone repair in osteonecrosis. Bone marrow also contains
endothelial progenitors. Endothelial progenitor cells (EPCs) are spindle
shaped cells capable of differentiation into a mature endothelial pheno-
type. These cells can be isolated from bone marrow and peripheral
blood. Their role in angiogenesis and neovascularization has been studied
extensively, and positive effects on blood vessel formation after
transplantation have been reported. Interestingly, Feng and colleagues
[8] recently reported significantly decreased numbers of circulating
EPCs and MSCs in patients with diagnosed osteonecrosis in comparison
with a healthy control group. Furthermore, EPCs of patients with osteo-
necrosis exhibited impaired migratory capacities and increased cellular
senescence, resulting in reduced angiogenesis in vitro [8]. In addition to
the generation of new capillaries, the growing endothelium enhance
mobilization and growth of mesenchymal progenitors through the
angiopoietin 1–Tie2 pathway. This pathway generates pericytes and
vascular mural cells required for new vessel growth and stabilization
[14].

Techniques for treating hip osteonecrosis with bone marrow
concentration

Bone marrow aspiration and cell harvesting

Bone marrow can be collected [2] from either the anterior or poste-
rior locations of the iliac crest. For a supine patient, the target for har-
vesting will be the anterior iliac crest. Collection of bone marrow from
the iliac crest is accomplished by the use of a single beveled, aspirating
needle. A standard 10-cm$^3$ syringe [15] should be used to obtain the
bone marrow aspirate (BMA). Immediately prior to insertion, the nee-
dle and aspiration syringe are prepared by rinsing with a heparin solu-
tion. Manually advance the needle between the inner and outer walls of
the iliac crest to a depth of 5 cm. Aspiration is initiated with a rapid pull
of the plunger. Syringes are exchanged as they become approximately
half full, without adjusting the level of the needle. The needle is
turned 45° during successive aspirations to reorient the bevel, there-
by affording aspiration from the largest possible area in the crest.
After one full 360° turn, the needle is moved 2 cm towards the surface
through the same insertion site and successive aspirations are per-
formed. The needle is always turned 45° after each aspiration. Bone
marrow aspirate is richer in stem cells when it is aspirated in small
volumes. This technique reduces dilution by peripheral blood. Aspirates
are pooled in sterile blood unit bags containing cell culture medium
(without any additional proteins present) and anticoagulant solution.
Pooled aspirates are filtered to separate cellular aggregates and fat, and
the mononuclear fraction is separated by standard procedures in
order to concentrate the cell preparation prior to injection. A bone
marrow concentrate (BMC) can be achieved by several commercial,
point of care systems specifically designed for concentrating bone
marrow aspirate in the operating room.

Intra-osseous injection of BMC

Patients can be placed on two image intensifiers on a
C-arm. The decompression is done with a percutaneous approach
using a 4 mm diameter trephine (trocar). The bone marrow is injected
into the femoral head using a small trocar [16,17]. The instrument is in-
roduced through the greater trochanter, as in a conventional core
decompression. The instrument position in the femoral head and in
the necrotic segment is monitored with biplane fluoroscopy. If the
plain radiographs show little evidence of necrosis, the preoperative
MRI scans can be used together with the image intensifier views to
determine the site of the lesion.

The complexity of the ideal place of cell injection has been discussed
in a previous paper [16]. From a theoretical point of view, the injection
should be performed in the dead center, at the reactive periphery, and
in the living part of the bone. In practical the adjunction of contrast prod-
uct to the bone marrow concentrate has demonstrated that, whatever
the point of injection, as soon as the volume of injection is more than
10 ml, there is diffusion of the bone marrow in the whole femoral
head, both dead and living part [16].

Clinical outcomes in treating hip osteonecrosis with bone marrow
concentration

Hernigou and Beaujean have reported on the treatment of 189 hips
in 116 patients with autologous BMC, with a follow-up of between
five and ten years [9]. Satisfactory results were achieved in a majority
of patients as seen in an improved Harris hip score, radiographic assess-
ment and absence of progression to total hip arthroplasty (THA). Patient
prognois was highly correlated to the stage of disease and the number
of progenitor cells injected. Better outcomes were observed when
patients were operated on before collapse and who received a greater
number of BMC injections. While this study contained no control
group, comparative studies [18] were reported where the beneficial
effect of the injected bone marrow concentrate was demonstrated
when compared to the standard of care core decompression alone on
the contralateral side. The difficulty of comparative studies is that they
need to be performed in patients with bilateral osteonecrosis so that
the osteonecrosis has the same etiology and the injection of bone mar-
row performed at the same time. Gangji and Hauzeur [19,20] conducted
a controlled prospective study comparing the post-operative outcome
of core decompression with core decompression followed by the injec-
tion of a bone marrow concentrate in early-stage AVN (ARCO stage I or
II) demonstrating advantage for the group with bone marrow injection.
Recently, Gangji and colleagues [21] reported on the 5-year follow-up of
19 patients (24 hip joints). In this prospective, double-blinded trial,
eight out of 11 hip joints in the core decompression group revealed a
disease progression with structural disintegration of the subchondral
bone. Only three out of 13 joints in the bone marrow concentrate
group showed progression of the disease. Despite these promising
results, the clinical value of these studies is limited because of short-
term follow-up periods and low case numbers.

In 2008, Hernigou et al. [22] retrospectively analyzed a large case
series covering 534 hips in 342 patients with ONFH that had been
treated with autologous BMC transplantation. The results were positive.
They showed that the volume of necrosis decreased from 26 cm$^3$ to
12 cm$^3$ in 371 patients with an average follow-up of 12 years. Only 94
patients progressed to THA. The authors concluded that the best indica-
tion for cytotherapy of ONFH was during the pre-collapse stage when
the hip was symptomatic.

Others [23–26] have reported on the combination of cytotherapy and
conventional therapy, including core decompression and autol-
ogous bone grafting, as a treatment for ONFH. Jones and Yang [29]
reported the effect of bone marrow mononuclear cells on vasculari-
zation and bone regeneration in steroid–induced osteonecrosis of
the femoral head. Yamashita and colleagues [25,26] used autologous

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bone marrow mononucleated cells (BMMNCs) that were transplanted into the affected area of one hip using interconnected porous calcium hydroxyapatite (IP-CHA) in two patients, while the other hip was simultaneously treated with transstrochanteric rotational osteotomy. This case report documents the potential of BMMNCs with IP-CHA for bone repair at the lesion of osteonecrosis of the femoral head. Liu and co-workers [24] recently reported a study where patients with ONFH underwent core decompression and implantation of nano-hydroxyapatite/polyamide bone filler with or without BMMNCs. Granular porous medical grade nano-hydroxyapatite/polyamide composite bone filling material was soaked in the concentrated BMMNC solution until completely absorbed (2 min), after which the bone filling particles/BMMC mixture was implanted in the bone tunnel. Repeated filling and compacting of the particles was performed using a pushing bar to ensure even loading. All patients with stage IIIA ONFH underwent reduction of the collapsed femoral head with success. Wang et al. [27] treated ONFH by core decompression and implantation of autologous bone marrow concentrate. The marrow aspirated from the iliac crest underwent concentration and the BMC was implanted in 59 hips in 45 patients with ONFH of ARCO stages I–IIIA. The average Harris hip score improved from 71 to 83 and only 11.9% of the treated patients required hip arthroplasty after an average follow-up of 27.6 months. Kang et al. reported the results of the combination of autologous, iliac cancellous bone and implantation of autologous bone marrow cells for ONFH, showing good clinical results in a short-term average follow-up period of 32 months [28]. Clinical outcomes are mainly analyzed with hip score and collapse. Often, the exact numbers of hips at the different stages ARCO are not precise nor the number of failure within stages in the different papers due to different classifications in different countries. The restoration of original NMR signals of a living bone marrow could be a standard in assessing the effectiveness of treatment. However, it appears that there are different variations in repair of the abnormal tissue (normal tissue; ossification; fibrous tissue) in the absence of associated scaffolds; furthermore, when scaffolds are used (hydroxyapatite), the presence of bone substitute remains present in the femoral head during a long time (several years), and of course act as an artifact to evaluate the exact repair. So, at this moment most of the clinical studies keep as outcome the absence of collapse during the evolution.

Treating ONFH with ex vivo expanded autologous bone marrow-derived MSCs

The rationale of ex vivo expansion of bone marrow-derived MSCs is to increase the concentration and the number of cells that can be injected. Within the bone marrow, MSCs are adherent cells that contribute to the niche of non-adherent hematopoietic stem cells (HSCs). Although MSCs occupy only 0.01% of the total population of nucleated cells in bone marrow, in vitro they have a high expansion ratio of over 1 thousand-fold, while maintaining multi-lineage differentiation capacity [29,30]. They routinely are expanded for over ten passages in media containing defined components. Techniques to purify MSCs include gradient centrifugation and selection by the adherent mononuclear cell population on tissue culture-treated Petri dishes or MSCs include gradient centrifugation and selection by the adherent mononuclear cell population on tissue culture-treated Petri dishes or MSCs include gradient centrifugation and selection by the adherent mononuclear cell population on tissue culture-treated Petri dishes or by immunophenotyping. High MSC yields can be achieved by in vitro culture within 2–3 weeks [29,30]. However, these expansion techniques result in daughter cells that have reduced differentiation capacity and impaired cell function including gradual accumulation of senescence-related markers and an increased potential for tumor transformation. The culturing process itself is slow and expensive and requires industrial-scale good-manufacturing practice (GMP)-compatible cell expansion facilities and a two-stage surgery for tissue procurement and implantation [30].

For example, Gangji and Hauzeur [31] reported on two patients with ONFH who were treated by injection of a suspension of autologous cultured bone marrow stromal cells (BMSCs). Osteoprogenitors and osteoblasts from bone marrow were separated and expanded in vitro, and then injected into the necrotic zone after differentiation. Pain reduction, necrotic lesion decrease and functional improvement were reported, and only minor side-effects were observed. The use of cultured autologous cells also has been reported in combination with a variety of matrices, such as ceramics, collagen sponges, hydrogels, and biodegradable polymers. Core decompression offers the opportunity to deliver such cell-laden biomaterials to the necrotic area. Use of a matrix to achieve tissue neoformation requires that scaffolds ideally undergo degradation processes resulting in biocompatible by-products, while also remaining injectable and non-toxic for MSCs. Kawate and colleagues [23] reported the treatment of three patients with advanced stages of cortisone-induced osteonecrosis (Steinberg stage III or IV) with a vascularized fibular graft combined with a synthetic β-TCP ceramic and bone marrow-derived, expanded MSCs. Four weeks prior to elective core decompression, 15 mL of bone marrow aspirate was taken from the iliac crest, and MSCs were isolated and expanded in autologous serum. After 10 days, MSCs were seeded onto the β-TCP granules and cultured for 2 weeks. After core decompression, the defect was filled with the MSC-seeded β-TCP granules and a vascularized fibular graft was transplanted. During the 34-month follow-up, no progression of the AVN was reported. A similar approach to treat AVN with autologous stromal cell-seeded β-TCP granules was presented by Liu, Zhao, and Rackwitz [32–34].

Allogeneic bone marrow-derived stem cell therapy of ONFH by intravenous delivery

Another unique advantage of MSCs is their potential for allogeneic 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, although MHC II expression could be induced by IFN-γ stimulation [29,30]. In addition, MSCs lack the expression of co-stimulatory molecules that activate T cells, including CD40, CD80 and CD86. MSCs have immunomodulatory effects of inhibiting the proliferation of T cells and B cells. Hernigou and colleagues [35] previously reported on the use of allogeneic 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 four years. The transplantation was performed in February 1992 after administration of a conditioning regimen of busulfan (16 mg per kilogram of body weight), cyclophosphamide (200 mg per kilogram of body weight), and total lymphoid irradiation in order to suppress the immune response and to eliminate hematopoietic precursors. The bone-marrow donor was an HLA-identical sibling for whom a mixed-leukocyte culture was non-reactive; the donor was heterozygous for sickle-cell anemia. The marrow was infused intravenously for one-half hour, beginning 48 h after the last infusion of cyclophosphamide. The dose of nucleated marrow cells that was infused was 200 million per kilogram of body weight. The patient had total repair of his osteonecrosis. The use of allogenic instead of autologous MSCs for the treatment of AVN appears attractive because of logistic and economic advantages given that these cells might be available as an ‘off the shelf’ product.

One concern of transplantation of MSCs is whether they can proliferate in the target tissues. At present, it is recognized that stem and progenitor cell homing is accomplished in two ways: cell necrosis after trauma results in release of a variety of signal molecules, and/or stem cells migrate into peripheral blood and target tissue where specific receptors or ligands are expressed. Progenitor cells circulate among tissues, and are able to migrate to the injured tissues once injury occurs.

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Stem and progenitor cell homing is a complicated process in which a wide variety of molecules are involved. Once tissues become ischemic, progenitor cells in circulation are adherent to the vascular endothelial cells, migrate across the endothelial cells, and finally reach the ischemic sites.

Recently the ability of circulating MSCs to home to an ischemic femoral head was confirmed by Li [36] in animals. In order to confirm that intravenously implanted allogenic MSCs can migrate to the femoral head, Li conducted two experiments. First, the distribution of allogenic MSCs in live nude mice was dynamically monitored after MSC injection. Results showed that MSCs can not only migrate into the femoral head, but also were retained in the femoral head for a relatively long time. Second, the sections of bone marrow, lungs, liver, and normal and necrotic femoral heads of rabbits with MSC transplantation were observed under fluorescence and light microscope. Results revealed that the amount of MSCs in the necrotic femoral head was higher than that in the normal femoral head, liver and lungs, indicating that femoral head ischemia or necrosis could induce MSCs to migrate into injured femoral heads. This means suggests that MSCs used to treat a pathologic tissue such as dead bone probably will remain in situ. Similarly, Yan et al. [37] demonstrated that BMMSCs could survive and expand in the ischemic environment of the femoral head up to 12 weeks after transplantation, showing significant proliferation of the BMMSCs in situ. In addition, Yan et al. [37] demonstrated that transplanted MSCs can differentiate into osteoblasts in the osteonecrotic region of ONFH, and hypoxia can stimulate MSCs to secrete angiogenic factors resulting in increased angiogenesis, which also could contribute to the bone repair process. Some papers have described that only 2% to 10% of allogenic stem cells can survive in mice after one month. However, one month in a mouse corresponds to several years in a human according to the metabolism and time of life difference; and 2% of the number of stem cells that are usually injected is equivalent to the normal number of stem cells present in a tissue.

ONFH treatment via intraarterial delivery of BMC and mononuclear cells

Mao [38] investigated the efficacy and safety of targeted delivery of autologous bone marrow enriched with mesenchymal stem cells (BMMSCs) via the medial circumflex femoral artery in the treatment of ONFH. The intra-arterial delivery of progenitor cells has been used in the treatment of vascular and cardiac disease. The targeted intraarterial delivery of autologous BMMSCs could be a minimally invasive strategy for the treatment of ONFH, since it avoids core decompression. The medial circumflex femoral artery is the main vessel which nourishes the femoral head. Furthermore, different studies have demonstrated pathological aspects of this artery in osteonecrosis. Therefore, intravascular infusion of mesenchymal stem cells could be applied to treat osteonecrosis, with the advantages of minimal invasiveness and injection of the cells directly in the vessels. Sixty-two patients (78 hips) with ONFH were recruited in this study. All of these patients were treated with BMSC perfusion via medial circumflex femoral artery. The concentrated BMMSCs (30–60 mL) were obtained from autologous bone marrow (100–200 mL) harvested from the anterior iliac crest and then were intra-arterially perfused into the femoral head. The average original volume of bone marrow harvested from each patient was $127.42 \pm 44.97$ mL, and was concentrated into $44.59 \pm 15.74$ mL after density gradient centrifugation with Percoll. A follow-up on the patients was done at the end of five years, and 92.31% (72 of 78) of hips achieved a satisfactory clinical result while only 6 hips (7.69%) progressed to clinical failure and required hip arthroplasty. This work suggests that autologous BMMSC perfusion via the medial circumflex femoral artery is a safe, effective and minimally invasive treatment strategy for early-stage ONFH.

Theoretical assessment of MSC levels in treating ONFH

As indicated in the review of clinical outcomes with BMC treatment of ONFH, there is probably no ideal number of MSCs that guarantee repair in osteonecrosis, and, in fact, the critical number could depend on the cause of osteonecrosis. We can only speculate on a threshold value according to the bone remodeling turnover rate and the number of MSCs that are present in a normal femoral head. A femoral head with a volume of 50 cm$^3$ will contain approximately 35,000 MSCs (see above), which may be considered as the target number in order to calculate the number of MSCs to load in an osteonecrotic femoral head to achieve the same number of MSCs present before osteonecrosis. Now considering the volume of the osteonecrosis [39,40], if a patient has an osteonecrotic lesion of 17 cm$^3$ and a femoral head of 50 cm$^3$ (thus the lesion is roughly 1/3 the head volume), the minimum number of MSCs to inject is roughly 12,000 MSCs (i.e., 35000/3). However, as illustrated above, there is a reduction in the level of MSCs in the area surrounding the osteonecrosis, so the number of MSCs to load will exceed 12,000.

It also is necessary to consider that only a portion of bone marrow cells will remain in the femoral head after implantation due to venous drainage. Homing studies have been performed by Hernigou and Beaujean [9] and Gangji [21] to determine this proportion. Both groups show that between 30% and 50% of labeled cells remained in the femoral head 24 h after implantation from a volume of 20 cm$^3$ to 40 cm$^3$. This proportion may vary according to the volume injected in the femoral head, since a larger volume may increase the proportion of cells lost by venous drainage, while a smaller volume might not fill the entire volume of the osteonecrotic lesion. Another point is that a part of the cells may be lost in the dead space of the trocar. So from a theoretical point of view, the number of MSCs to inject should be greater than 24,000 MSCs and probably situated between 24,000 and 35,000.

We also can calculate the number of MSCs that is necessary to repair a similar volume of tissue in a femoral head. Histologic observations [41] have demonstrated that the ratio of trabecular bone is 1:3 in the femoral head, with the remaining tissue being comprised of fat and hematologic cells. This would indicate that during the repair of a lesion of 18 cm$^3$ of femoral head, 1/3 of the repaired tissue is bone, which requires the repair of 6 cm$^3$ of bone during the healing process. Histologic observations and methods of quantitative histomorphometry pioneered by Frost [41] and Parfitt [42] have provided understanding of the functional and dynamic parameters associated with bone formation and remodeling at the tissue level. In adult bone remodeling, these processes of bone formation take place in the context of the basic multicellular unit (BMU) described by Frost. 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. The total matrix synthesis per osteoblast is approximately 6000–9000 μm$^3$, or 3–5 times its cell volume. Therefore it can be estimated that the volume of bone matrix made by one osteoblast is approximately 5000 mm$^3$. During the process of bone formation, some osteoblasts become embedded in the newly synthesized matrix as osteocytes. These osteocytes reside within cavities known as lacunae and interconnect with one another through multiple processes extending through an interconnected plexus of channels called canaliculi. Some osteoblasts also undergo apoptosis. The osteocyte density is reported to be greater in cancellous bone (0.000047 osteocytes/μm$^3$) than in cortical bone (0.000026 osteocytes/μm$^3$). As a first approximation, based on a mean bone matrix comprised of 33% cancellous bone, one can estimate the number of osteocytes in 1 cm$^3$ of cancellous bone to be in the range of 20 million. Given the measured number of 2500 progenitors per mL of prepared bone marrow concentrate [43], each progenitor must have divided a minimum of 12 or 14 times to obtain 1 mL of new bone, assuming that all the progenitor cells retained the ability to make trabecular bone (i.e., $2500 \times 2^{12} = 20$ million
osteoblasts). These calculations assume that all of the injected cells remain in place (which is not true) and that there are no other osteogenic cells recruited to the site of pathology (which is unlikely, since MSCs are known to recruit cells from adjacent tissue). An injection of 20 cm$^3$ of bone marrow concentrate will yield 50,000 MSCs (i.e., 2500 × 20). So the two different modes of calculation arrive at a similar minimum number of MSCs situated between 25,000 and 50,000. Of course, from a theoretical point of view the repair will be easier for a small osteonecrosis if the number of cells available from the iliac crest is the same, but this remains an unconfirmed hypothesis.

Variation in the levels of MSCs used in clinical studies

One of the major difficulties in comparing various studies in assessing the efficacy of MSC cell therapy for treating ONFH is that there are a variety of different methods available to count MSCs, which are not necessarily equivalent. For example, one of the methods is to focus on assessing concentrations of stromal clonogenic cells, as indicated by colony forming units-fibroblast (CFU-F). CFU-F assays depend on multi-day culturing in media that usually contains fetal bovine serum. However, variation in serum heat inactivation could significantly affect colony formation. The problem isn't aided by the use of non-heat-inactivated fetal bovine serum, since the resulting culture medium can show wide variability in the efficiency of CFU-F formation (colony-forming efficiency, CFE). Another technique is to count cells expressing surface markers that are thought to be characteristic of MSCs (i.e. their phenotype) to separate stroma cells from hematopoietic and endothelial progenitor cells. However, there is no specific marker that can identify MSCs from other cell types. MSCs express low levels of collagen types I and III and osteonectin and are, therefore, considered fibroblastic. These cells also co-express alkaline phosphatase, endoglin (CD105), and high levels of CD146, the latter being found at high levels in cells within CFU-Fs in vitro and of perivascular cells in vivo. MSCs generally express markers including STRO-1 (a stromal cell surface marker), CD29 (integrin α1), CD44 (receptor for hyaluronic acid and matrix proteins), CD105 (endoglin) and CD166 (cell adhesion-molecule), although the expression may vary with the state of the cell and can change during culturing. On the other hand, cultured MSCs do not express CD14 (monocyte surface antigen), CD34 (HSC surface antigen) and CD45 (leukocyte surface antigen). In some cases, such cell surface markers have been empirically used to isolate MSCs by fluorescence activated cell sorting or magnetic-activated cell sorting. However, this surface marker profile is not standardized for all MSCs owing to the heterogeneity of cell phenotype from various tissue origins and species. For example, CD34 appears to be expressed in MSCs derived from mice, but are absent in cultured human MSCs. Nevertheless, various combinations of these surface markers frequently are used for characterizing MSCs.

While it might be difficult to compare the number of MSCs used in the various clinical studies, due to variability in counting protocols, it is possible to assess the number of MSCs present in preparations obtained from crude bone marrow without concentration, bone marrow with concentration, and ex vivo expanded cells from bone marrow. In the absence of concentration of the bone marrow aspirate, it is clear that a femoral head osteonecrosis theoretically cannot be charged by injection into a femoral head is 10 mL. This means that without concentration, the number of MSC that can be loaded is between 5000 and 20,000, which is well below the number of MSCs in a normal femoral head as cited above.

When bone marrow aspirate is concentrated 5–10 fold, the level of available MSCs for loading is substantially increased. According to the concentration technique (between 5 and 10 times), and the volume of bone marrow aspirate, the number of MSCs that was injected as reported in the various clinical studies was between 25,000 and 200,000 MSCs. Phenotypic counting could result in a 10-fold higher cell count, but the weaknesses of this method have been described above.

While concentration of a patient’s bone marrow aspirate definitely will provide cell therapy preparations with higher concentrations of MSCs, nonetheless, the inherent patient-to-patient variability in MSC levels may limit the utility of the use of BMC. Variability in MSC content of a patient’s bone marrow aspirate could depend on a variety of factors, including difficulty of aspirating bone marrow, syringe volume and fill during aspiration, as well as intrinsic patient-dependent variation. For example, decreased levels of MSCs have been described in patients with corticosteroid-induced hip osteonecrosis, and alcohol-induced hip osteonecrosis. MSCs counts were shown to be abnormal in some hematological disorders and decreased in other conditions, such as tobacco-use. Progenitor cell counts in bone marrow aspirates also varied depending on the age of patient. As a result of these co-morbidities, there may be limitations in the therapeutic potential of the patient’s autologous bone marrow concentrate. However, in these cases, it is a standard practice to draw larger volumes of bone marrow prior to concentration.

One potential solution to address the inherent variability of MSC content in patients is to consider cell therapy based on a tissue engineering approach. 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 them onto 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. In some clinical studies in which autologous expanded MSCs were used, the authors failed to report the exact number of MSCs used in the treatment. However, usually the number of MSCs expanded after three weeks of tissue culture is about 5 million MSCs per mL. So according to the reported culturing time, ranging from ten days to three weeks, the number of cultured MSCs could range from 100,000 to 20 million cells.

Safety of injection of MSCs

Bone marrow aspiration and the technique of BMC injection with a small diameter (4 mm) trocar were not factors that limited rehabilitation or resulted in the prolonging of patient stays prior to discharge from the hospital, as has been observed after a standard of care open procedure for treatment of osteonecrosis through core decompression with a trocar of 8 mm diameter. According to Tocantins intra-osseous injection of a therapeutic substance was first described by Josefson in 1934, who administered campolon by direct intrasternal puncture to treat pernicious anemia. Intra-osseous injection of physiological serum into bone marrow for pediatric reanimation when the extent of shock was too severe for rapid venous access has been proposed by Wallden without complications. Intra-osseous injection of marrow in the femoral head as described here is based on the same principle. Given the permeability of bone tissue to liquid substances (as described by the physiologist Drinker in 1922), one of the theoretical criticisms of this technique might be a risk of fat embolism during intra-osseous infusion. Although no cases of fat embolism have been described in literature reports of intra-osseous infusion in pediatric reanimation, the risk may exist. Therefore, we use a filter to decrease the quantity of fat present in the BMC to a level that is in our experience insufficient to produce respiratory distress or to modify arterial O$_2$ pressure. Starting in 1990, we have performed this procedure on more than two thousand hip osteonecroses, and have observed a decrease in blood pressure during the injection for only two patients.

Bone marrow-derived mesenchymal stem cells (MSCs) may induce a tumorigenic process under special circumstances. Precisely how
regenerating engineered tissues may interact with cancer cells in vivo is currently unknown. There is speculation that bone marrow derived cells may contribute to cancer development by supporting tumor angiogenesis. Some tumors secrete chemotactic signals to mobilize stem cells from the marrow, and these cells have been shown to be incorporated into the vasculature of tumors in mice. While most studies of cell therapy have focused on the potential benefits of treatment, the safety profile of cell therapy is rarely addressed in detail because this therapy has not presented significant adverse events and the number of patients in individual trials or publications tends to be relatively small and usually with a short follow-up.

Therefore, to evaluate the risk of the development of progenitor cell-induced cancers Hernigou and co-workers [49] investigated the long-term risks for treatment site-specific cancers, as well as the incidence of cancer elsewhere in 1089 patients who received bone marrow concentrate progenitor cells to treat osteonecroses. The patients had follow-ups with radiographs and/or MRIs after their procedure at various time points. The follow-up period in the present study ranged from 5 to 22 years. No MRI evidence of tumorigenesis was observed at the re-implant sites. All had negative MRIs and negative radiographs (as read by both a radiologist and the orthopaedic surgeon) for any evidence of tumor formation at all measured imaging outcome endpoints. For comparison purposes, they used a 5-year minimum follow-up period, while experimental in vivo animal studies for tumorigenicity typically report on 90-day follow-up periods. MSCs replicate every 2–4 days in culture and if that growth was to continue at a similar pace after implantation, a small tumor even with a low growth rate probably would be discernible on MRI or radiographs within a few months. Concerning long term follow-up, while it is possible that local tumors may still form at some time beyond the 22 year follow-up period represented in our data, this possibility likely decreases at a geometric rate, and radiographs of the site of re-implantation are probably sufficient to analyze this risk in the very long term.

The secondary outcome was to evaluate the risk of cancer diagnosed in areas other than the re-implantation site during the follow-up period. The patients were followed for cancer incidence from the date of the first operation (1990) until death, or until 31 December 2011. The mean follow-up time was 12.5 years (range 5–20 years). The relative risk of cancer was expressed as the ratio of observed and expected number of cases in the population of the country, i.e. standardized incidence ratio (SIR), with statistics obtained from the French National Cancer Institute, and the age-adjusted cancer incidence calculated for patients in the study, Hernigou and co-workers [49] 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 higher number of MSCs.

Conclusion

Osteonecrosis is one of the most common hip diseases in young men and women. It is a progressive bone disorder characterized by bone cell death and structural deterioration of the bone tissue, leading to bone fragility and an increased risk of subchondral fracture or collapse. In particular, hip collapse constitutes a serious complication of hip osteonecrosis that is responsible for an average 10% of all total hip arthroplasties. The burden of hip osteonecrosis is increased in the young population with an average of 50% of hip arthroplasties performed annually are related to advanced osteonecrosis.

The standard of care treatment for osteonecrosis of the femoral head (core decompression) frequently is insufficient to stabilize the pathology, resulting in collapse and total hip arthroplasty. Since there is evidence that a patient’s osteonecrotic femoral head has fewer osteogenic progenitor cells and generally poor vascularity, the use of autologous cell therapy in the form of bone marrow concentrate has been evaluated. Substantial repair and stabilization of a necrotic femoral head have been achieved through the percutaneous injection of a patient’s own BMC in combination with core decompression. Cell number and activity of the injected cells are thought to play an important role in a therapeutic outcome.

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cultured with beta-tricalcium phosphate ceramics and free vascularized fibula.


