

Bone marrow aspirate and bone allograft to treat acetabular bone defects in revision total hip arthroplasty: preliminary report

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Abstract. – OBJECTIVES: The safety and effectiveness of autologous mesenchymal cells for treating bone defects in humans is still uncertain. The present study presents a new technique consisting of allogeneic bone grafting enriched with bone marrow concentrate to treat acetabular bone defects resulting from aseptic loosening of the acetabular cup after total hip replacement.

PATIENTS AND METHODS: Five adult patients were included in the study. Prior to surgery, patients were tested for antibodies to common pathogens. Treatment consisted of bone allogeneic scaffold seeded with bone marrow mesenchymal cells harvested from the iliac crest and concentrated using an FDA-cleared device. Clinical and radiographic follow-up was performed at 1, 3, 6, and 12 months after surgery. To assess viability, morphology, and the immunophenotype, bone marrow nucleated cells were cultured *in vitro*, then tested for sterility and evaluated for the possible replication of adventitious viruses.

RESULTS: In 4 of 5 patients, both clinical and radiographic healing of the bone defect together with bone graft integration was observed at the mean time of 3.5 months. Mean follow-up was 2 years. One patient failed to respond. No post-operative complications were observed. Bone marrow nucleated cells were enriched 3.8-fold by a single concentration step. Enriched cells were free of microbial contamination. The immunophenotype of adherent cells was compatible with that of mesenchymal stem cells. No viral reactivation was observed.

CONCLUSIONS: Allogeneic bone scaffold enriched with concentrated autologous bone marrow cells obtained from the iliac crest, may represent a good alternative to treat acetabular bone defects observed in revision hip arthroplasty.

Key Words:

Bone marrow, Bone marrow concentrate, Mesenchymal stem cells, Bone defects, THA; Revision THA.

Introduction

In the last two decades, the number of primary and revision total hip arthroplasties (THA) has progressively increased in Europe, North America, and Australia¹⁻⁵. Kurtz et al¹ estimated that THA revisions in the United States (US) will grow by a stunning 137% from 2005 (40,800 procedures) to 2030 (96,700 procedures). This increase could determine a revision burden, defined as the rate of revision THA over the total number of THA performed, of 14.5%¹. One of the greatest problems that an orthopaedic surgeon must face when revising a failed THA is the loss of bone stock. For this reason, grafting with donor allograft became particularly popular over two decades ago^{6,7}. In time, other grafting techniques have been presented to compensate for diminished bone stock, such as autograft, xenografts, and ceramics⁸⁻¹⁰. Unfortunately, these techniques present limitations: autograft has a limited availability¹¹ and is associated with donor site morbidity¹², xenografts do not osseointegrate as well as allografts and may cause infections¹³, ceramics are scarcely osseointegrative and have scarce resistance to load^{14,15}. Therefore, grafting with allograft currently appears to be one of the better choices to reconstruct acetabular bone defects. However, one of the problems with allograft is the lack of osteoprogenitor cells. Giannoudis et al provided evidence that in order to achieve bone formation and healing, four parameters have to be respected: utilization of growth factors, scaffolds, mesenchymal stem cells, and stable mechanics¹⁶. In orthopaedic surgery, platelet-derived growth factors and bone morphogenetic proteins (BMPs) have been used to stimulate bone regeneration in a variety of clinical conditions, including long bone non-unions, spinal fusion surgery, and repair of symptomatic posterolateral lumbar spine non-unions^{17,18}. Bone marrow mesenchymal stem cells (MSCs) are multipotent

cells^{19,20} that have the potential to proliferate and differentiate into osteoblasts, chondroblasts, odontoblasts, adipocytes²¹. MSCs are of interest for clinical applications, since they can be easily obtained from bone marrow aspirates. In order for bone healing to occur, the implanted cells must differentiate into osteoblasts, secrete matrix constituents and differentiate into osteocytes. Despite extensive experimental knowledge^{19,22-27}, applications of adult MSCs for treating bone defects are scarce. The ideal cellular therapy protocol for bone regeneration should include isolating, expanding in vitro, and differentiating progenitor cells before re-implantation within an adequate extracellular matrix. Nonetheless, ethical and legislative considerations strictly regulate these procedures²⁸⁻³⁴. In light of this, autologous harvesting and immediate implantation of MSCs represents an appealing and simple technique. This type of approach could eliminate culture-derived problems, including cell aging, cell reprogramming, microbial contamination/infection and non-autologous culture components³⁵. To guarantee safety of cell therapy, US Pharmacopeia recommended the use of simple procedures whenever applicable³¹.

We present a new approach consisting of allogeneic bone graft seeded with autologous MSCs to treat cavitary acetabular bone defects in revision THA.

Patients and Methods

From January 2009 to August 2010, 5 patients – 3 males (70%) and 2 females (30%) – affected by aseptic loosening with contained acetabular defects undergoing revision THA were included in the study. Patients were excluded if they presented with any of the following: (1) aseptic loosening of the femoral component; (2) local or systemic infection; (3) severe metabolic and autoimmune disease; (4) antineoplastic therapy.

Patients were treated at the Department of Trauma and Orthopaedic Surgery, University of Insubria, Varese, Italy. Approval of the Hospital Ethics Committee and the written informed consent of patients were obtained for all cases. The mean age of patients at the time of surgery was 73 years (range, 66-77 years). Prior to surgery patients were tested for serum antibodies to common pathogens (*Treponema pallidum*, human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and parvovirus B19) (Table I).

Table I. Preoperative serological evaluation of patients.

Patient	Age	Gender	Treponema pallidum Total Ig	HIV Total Ig	HCV IgG	HBV		Parvovirus B19		EBV			CMV	
						HBsAg	Anti-HBc	IgG	IgM	IgG VCA	IgG EBNA	IgMVCA	IgG	IgM
1BA	66	M	-	-	-	-	-	+	-	+	+	-	+	-
2FM	74	F	-	-	-	-	-	+	-	ND	ND	ND	ND	ND
3FV77	M	-	-	-	-	+	+	-	+	+	-	ND	ND	-
4BA	75	F	-	-	-	-	-	+	-	+	+	-	+	-
5FL73	M	-	-	-	-	+	-	-	+	+	-	ND	ND	-
No. positive/No. tested			0/5	0/5	0/5	0/5	2/5	4/5	0/5	4/4	4/4	0/4	2/2	0/2

ND: not detected.

Harvest and Processing of Bone Marrow Cells

The SmartPREP-2 Bone Marrow Aspirate Concentrate System (Harvest Technologies GmbH, Munich, Germany) was used for concentrating nucleated bone marrow cells. Fresh bone marrow aspirate (BMA) is obtained from the posterior iliac crest using a 6 lumen Jamshidi type trocar needle and 20 ml syringes pre-flushed with Na-heparin (1,000 units/ml). About 60 ml of aspirate are necessary. BMA is then concentrated using the SmartPREP-2 system in the operating suite. During the centrifugation phase, red blood cells (RBCs) are separated from nucleated cells, platelets and plasma. The concentration step is accomplished in 15 minutes. Bone marrow aspirate concentrate (BMAC) contains unchanged proportions of myelocytes, granulocytes, lymphocytes, monocytes, proerythroblasts and erythroblasts as compared to the initial bone marrow aspirate³⁶. BMAC (about 10 ml) is then transferred, under sterile conditions, to the surgical field. BMAC and homologous bone graft are gently mixed with a spatula.

Surgical Methods

The surgical technique requires exposure of the bone defect via a posterolateral approach, swabs to be sent for microbiological cultures, debridement of the bony surface until viable tissue is visible, followed by grafting of allogeneic bone (Bone Bank; Istituto Ortopedico G. Pini, Milan, Italy) enriched with BMAC. The graft was impacted by reverse reaming. The acetabular components were replaced with a TMT cup (Zimmer Trabecular Metal™ Technology; Zimmer, Warsaw, IN) plus screws. Mechanical stability was always thoroughly evaluated and accounted for. At the end of the surgical procedure, 2 ml of BMA and approximately 0.5 ml of BMAC were submitted to the Microbiology laboratory for the following procedures: (1) pre- and post-concentration cell count; (2) sterility tests; (3) culture of nucleated cells to assess cell morphology, replication, and differentiation; (4) assessment of virus-induced CPE and of adventitious viruses.

Preoperative Serological Status of Patients

Serum specimens taken from patients before surgery were tested for multiple pathogens. Antibodies to HIV, HBV, HCV, CMV, EBV, and parvovirus B19 were tested using enzyme-linked

immunosorbent assays (ELISA; Diasorin, Saluggia, Italy; Biotrin, Dublin, Ireland). Antibodies to *T. pallidum* were assayed against recombinant antigens (Diasorin, Saluggia, Italy).

Bone Marrow cell Cultures

Two samples of bone marrow (pre- and post-concentration) were obtained in heparin tubes from each patient. Blood cell counts were obtained using routine analyzers (Sysmex; Norderstedt, Germany). Nucleated cells were isolated by centrifugation on two different Ficoll-Histopaque gradients (1.077 and 1.199 g/ml). Cell viability was evaluated by the trypan blue exclusion method. Cell suspensions were plated in 75 cm² tissue culture flasks using Mesenchymal Stem Cell Growth Medium (Lonza; Basel, Switzerland) plus basic fibroblast growth factor (bFGF; 5 ng/ml) and incubated at 37°C in air with 5% CO₂. Stromal cells adhered to plastic within 48 hrs from plating; then cell replication started. Upon reaching confluency in 5-7 days, adherent cells were harvested using trypsin-EDTA and replated for subsequent passages. Supernatant was collected from each culture at different times, clarified by low-speed centrifugation, aliquoted and frozen at -70°C before processing.

Cultures were observed at 3-day intervals using a phase-contrast microscope with a digital camera in order to evaluate cell morphology and the possible development of CPE. Images were acquired with a 10X, 20X, and 40X objective and recorded using a computerized image analysis system (Image DB; Amplimedical, Mira, IT).

Sterility tests, Mycoplasma Detection, and Detection of Adventitious Viruses in Cultured Bone Marrow cells

Aliquots of BMA, BMAC, and bone marrow cells cultured for 2-4 weeks were inoculated into aerobic and anaerobic blood culture vials (BACTEC™ Plus Aerobic/F* and Plus Anaerobic/F* Vials; Becton Dickinson, Franklin Lakes, NJ, USA) and incubated for 14 days. Cell culture supernatants were also tested for *Mycoplasma* spp. using a commercial kit (MycAlert; Lonza Rockland, ME, USA) based on enzymatic ATP-conversion combined with luminescence measurement. The test can detect a wide range of *Mycoplasma* species.

At the end of the 4 week culture period, cultured bone marrow cells were tested for adventitious viruses by polymerase chain reaction (PCR).

Characterization of Cultured Bone Marrow Cells

The surface expression of differentiation markers was evaluated by flow cytometry (FACSCalibur; Becton Dickinson Biosciences, Franklin Lakes, NJ, USA) and immunofluorescence. The following mouse monoclonal antibodies were used: CD105, CD73, CD90 (expected expression by MSCs), CD45, CD34, CD14 (expected lack of expression by MSCs). Cultured adherent cells were washed with PBS, detached by scraping, and counted. Cell suspensions (approximately 10^6 /ml in FACS buffer) were dispensed in sterile tubes (0.3 ml/tube) and incubated on ice with antibodies directed against different markers. As secondary antibody, FITC-labeled goat anti-mouse was used. Two negative controls were stained with FITC secondary antibody: unstained cells and cells incubated with a non-relevant mAb (anti-HIV p24).

Follow-up of Patients

According to the routine protocol used at our Department of Trauma and Orthopaedic Surgery, each patient was evaluated clinically and radiographically (2 plane x-rays) at 1 month post-op, 3 months post-op, 6 months post-op, and 12 months post-op. Osseointegration was radiographically evaluated according to the 5 criteria by Moore et al³⁷: 1) absence of radiolucent line; 2) presence of superolateral buttress; 3) presence of medial stress-shielding; 4) presence of radial trabecular pattern; 5) presence of inferomedial buttress. The presence of at least 3 signs is suggestive of 90% sensitivity and 77% specificity³⁷. Adverse events experienced by patients during the study were recorded. At one year after surgery, patients completed the Satisfaction Scale for Joint Replacement Arthroplasty³⁸. The scale consists of 4 questions rating patient satisfaction with hip replacement in terms of pain relief, ability to perform activities of daily living (ADLs), ability to partake in leisure activities, and overall satisfaction. A total satisfaction score ranges from 0-100 (full dissatisfaction to full satisfaction). Results were analyzed using observational statistics.

Results

Serological Evaluation Before Surgery

As shown in Table I, no positivity against *T.pallidum*, HIV, and HCV was detected. Anti-

HBc antibody was found in 2 of 5 patients (40%), no one of which was HBsAg-positive. IgG to parvovirus B19 was detected in 4 of 5 patients (80%); IgG against EBV and CMV were present in all patients (100%). These data substantiate the risk for surgeons of accidental infections by HBV, EBV, CMV, and parvovirus B19.

Nucleated cells in Bone Marrow Aspirates

As shown in Table III, cell counts performed before and after concentration showed that the procedure reduced RBC numbers by almost a half, and concentrated nucleated cells and platelets by almost 4 times. Mean 716 million nucleated cells (range, 470-970 millions) were implanted in each patient.

Cell Culture of Bone Marrow Aspirates: Sterility Tests and Phenotypic Characterization of Adherent Cells

Nucleated cells of BMA were cultured for 4 weeks. Two days after plating, a mixture of fibroblast-like and hemispherical adherent cells started growing and formed distinct colonies that progressively transformed into monolayers of adherent fibroblast-like cells with rare hemispherical adherent cells (Figure 1 a-d).

Sterility tests performed on cultures incubated for 4 weeks showed no bacterial or fungal contamination; mycoplasma contamination was also absent (Table III). Cell viability was 95-99%. Cytofluorimetry and immunofluorescence showed that adherent bone marrow cells from all patients were negative for the CD45 haematopoietic marker, for CD34 (endothelial marker), and for CD14 (monocyte marker). Cultured cells from most patients expressed the surface markers CD73, CD90, and CD105. The results are in agreement with the criteria of the International Society for Cellular Therapy for defining multipotent mesenchymal stromal cells³⁹.

Adventitious viruses were evaluated by observing the development of CPE in cell cultures and by amplification of viral genomes (Table IV). After 2-4 weeks incubation, all samples were sterile for the tested pathogens.

Clinical Outcome

Clinical data and number of implanted cells are summarised in Table V. No patient was lost at follow-up. Mean follow-up was 25 months (range, 16-36 months). There were no further revisions at the time of this publication. There

Table II. Bone marrow aspirates from patients.

Patient	RBC ($\times 10^6/\mu\text{l}$)			Nucleated Cells ($\times 10^3/\mu\text{l}$)			PLT ($\times 10^3/\mu\text{l}$)		
	Pre-Concentration	Post-Concentration	Concentration factor	Pre-Concentration	Post-Concentration	Concentration factor	Pre-Concentration	Post-Concentration	Concentration factor
1ba	4.20	1.80	0.43	20.10	94.40	4.70	75.00	301.00	4.01
2FM	2.88	1.61	0.56	15.20	47.47	3.12	223.00	823.00	3.69
3FV	4.24	2.21	0.52	20.69	96.32	4.66	48.00	317.00	6.60
4BA	3.14	1.14	0.36	15.81	69.45	4.39	230.00	948.00	4.12
5FL	3.20	1.16	0.36	22.20	51.59	2.32	66.00	165.00	2.50
Mean			0.45			3.8			4.2
Standard deviation			0.09			1.1			1.5

RBC: red blood cells; PLT: platelets.

Table III. Sterility tests and phenotypic characterization of adherent cells cultured from bone marrow aspirates.

Patient	Sterility ¹	Mycoplasma detection ²	Cell viability (%) ³	Adhesion to plastic ⁴	Cell morphology ⁴	Surface markers of adherent cells ⁵						
						CD34	CD45	CD14	CD73	CD90	CD105	CD105
1BA	+	-	95	+	80% fibroblast-like; 20% hemispherical adherent cells	-	-	+	+	ND	+	+
2FM	+	-	98	+	90% fibroblast-like; 10% hemispherical adherent cells	-	-	ND	+	-	+	+
3FV	+	-	99	+	Fibroblast-like	ND	-	-	-	+	+	+
4BA	+	-	96	+	Fibroblast-like	ND	-	-	+	ND	+	+
5FL	+	-	99	+	Fibroblast-like	-	-	-	+	+	+	+
No. Positive/No. Tested						0/3	0/5	1/4	4/5	2/3	5/5	5/5

ND: not detected; 1. Sterility tests according to criteria of the US Pharmacopeia (USP-2011). +: positive; -: negative (after 4 week culture). 2. Mycoplasma detection as in the Methods Section. +: positive; -: negative (after 4 week culture). 3. Cell viability evaluated by the Trypan blue exclusion assay (after 4 week culture). 4. Adhesion to plastic and cell morphology evaluated by phase-contrast microscopy.

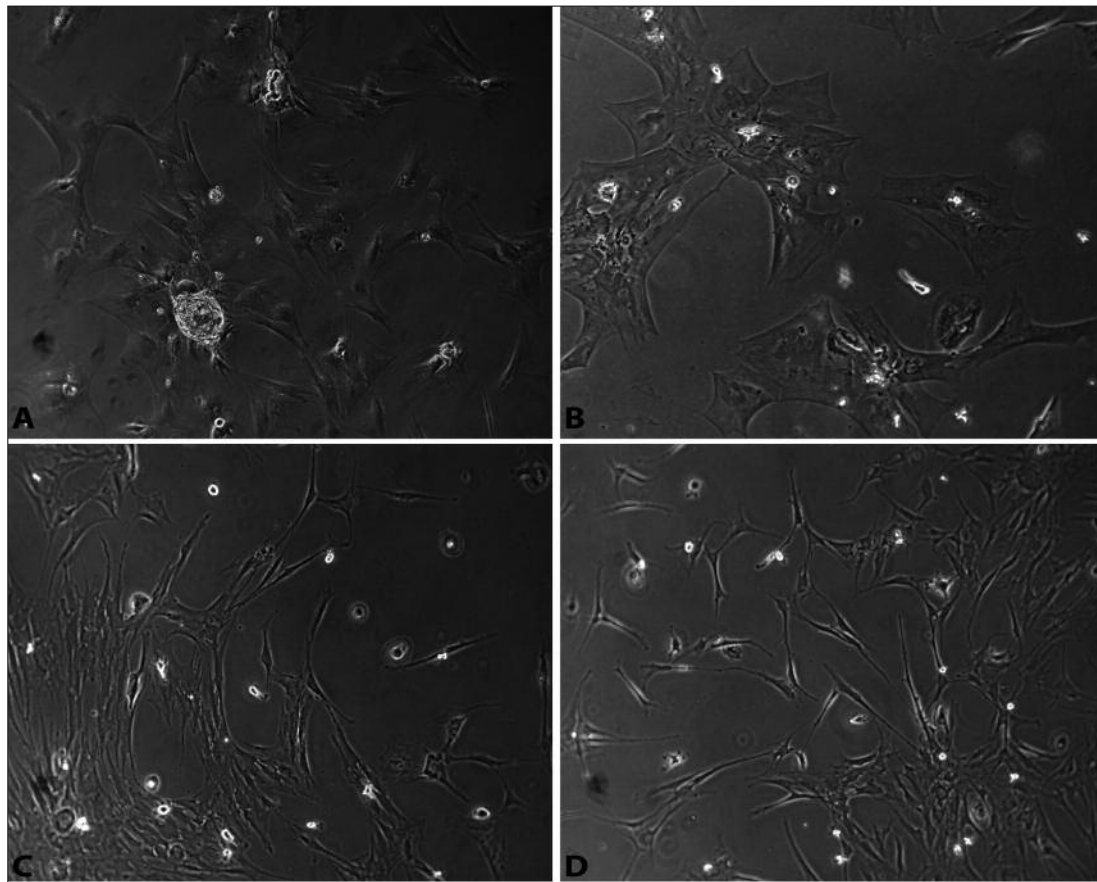


Figure 1. **A**, and **B**, Cultures of adherent bone marrow cells 5 days post-plating. In both cases, most cells show fibroblast-like morphology (case 3FV, A; case 4BA, B. Original magnification 200×). **C**, and **D**, Cultures of adherent bone marrow cells 12 days post-plating. In both cases, mixtures of fibroblast-like and hemispherical adherent cells are present (case 2FM C; case 5FL, D. Original magnification 100×).

were no infections. As per Moore's radiographic criteria for osseointegration³⁹, at mean 3.5 months after surgical intervention (range 2-6 months) 4 of 5 cases (80%) showed at least 3

signs of bone ingrowth, full integration of the bone graft, and complete healing of the bone defect (Figures 2, 3, 4). There was no migration of the implanted cups. Heterotopic ossification

Table IV. Detection of viral agents in cultures of bone marrow aspirates.

Patient	Cytopathic effect in cultured cells ¹	Amplification of viral genomes ²					
		Parvovirus B19	EBV	CMV	HSV-1/2	HBV	HERV-K
1BA	—	—	—	—	—	—	—
2FM	—	ND	ND	ND	ND	ND	ND
3FV	—	—	—	—	—	—	—
4BA	—	—	—	—	—	—	—
5FL	—	ND	ND	ND	ND	ND	ND
No. Positive/No. Tested	0/5	0/3	0/3	0/3	0/3	0/3	0/3

ND: not detected. 1. Evaluation of cytopathic effect through serial examination by phase-contrast microscopy. 2. Detection of adventitious viruses by gene amplification (after 4 week culture). See the Methods Section.

Table V. Characteristics of patients.

Patient	Age at surgery (years)	Sex	No. of implanted bone marrow nucleated cells ($\times 10^6$)	Clinical and radiographic healing (months)
1BA	66	M	940	Failure
2FM	74	F	470	3
3FV	77	M	960	6
4BA	75	F	690	3
5FL	73	M	520	2

was observed in 1 patient (20%) (Figure 4). The patient was asymptomatic. Mean Satisfaction Scale score was 82 (range, 50-95). In one patient (20%) neither clinical nor radiographic healing was achieved. The failure concerned a patient (patient 1BA) affected by aseptic loosening of the acetabular cup in a total hip replacement, complicated by a voluminous haematoma of the anterior region of the thigh. One month after acetabular cup substitution, and treatment with bone graft and bone marrow concentrate, the patient presented with a recurrence of the thigh haematoma and partial resorption of the bone graft. Clinical tests, imaging studies, and microbiological assays over the following months failed to identify local or systemic infection. Furthermore, osteointegration of the new acetabular cup failed to occur. Suspecting subclinical infection, the patient underwent repeated surgery nine months after the last intervention. The hip prosthesis was removed and substituted with an antibiotic spacer. Microbiological and histological examinations of the haematoma and periprosthetic soft tissues were performed, which were all inconclusive.

**Figure 2.** Preoperative AP radiograph of the pelvis. Case 2FM.

Discussion

Bone-marrow derived autologous mononuclear cells in combination with biocompatible scaffolds can support osteogenesis in animal models⁴⁰⁻⁴⁴ and in humans^{28,45}. The differentiation of osteoprogenitor cells relies on the surrounding microenvironment, making the use of BMAC a potentially valid option for bone regeneration⁴⁶⁻⁴⁹. We present a small case series of 5 revision THA patients affected by cavitory acetabular bone defects, which proved to be successful in 4 of 5 (80%) of patients. The pre-

**Figure 3.** AP and lateral radiographs of the right hip following revision arthroplasty – 3 months postop. Case 2FM



Figure 4. AP and lateral radiographs of the right hip following revision arthroplasty – 3 months postop. Case 2FM.

sent study reports complete healing of the bone defects within 2-6 months of revision surgery in 4 of 5 patients.

Our work presents several limitations: it is a preliminary report, lacks a control group, and is not randomised. Despite encouraging clinical and radiographic results in 4 of 5 cases, our conclusions cannot still guarantee reproducible results in larger series of patients.

Despite symptomatic improvement and significant bone formation observed in 4 of 5 patients (80%), treatment with autologous BMAC in combination with allogenic bone graft was unsuccessful in one patient. The 66 year-old gentleman who did not respond to the revision THA represented a complex clinical scenario. Despite extensive preoperative and postoperative work-up, the underlying cause of the failure was never identified. However, in the remaining patients, the method proved to be safe both perioperatively and at over 2 years postoperatively. Moreover, the procedure did not have a significant impact on the total operating time. Bone marrow samples (before and after concentration) were consistently sterile. The cost of the procedure was relatively cheap, especially if compared to alternative materials or drugs (e.g., bone morphogenetic proteins). In addition, the morbidity associated with autologous bone graft transplantation (infections, seromas, hematomas, herniation of abdominal contents, vascular injuries, neurologic injuries, and iliac wing fractures) was abolished¹², and no side effect was observed. Compared to the *ex-vivo* expansion of autologous cells from

bone marrow, one-stage isolation has lower costs and eliminates the risk of infections attributable to the complex procedures required for cellular expansion⁴⁵.

Analysis of cell differentiation markers and virological studies show that large numbers of MSCs are easily obtainable from bone marrow aspirates of adult patients. Furthermore, another major advantage of BMAC is the elevated platelet concentration (about 373%) reported in our study. Activated platelets release granules containing tissue growth factors, cytokines and chemokines: platelet-derived growth factor (PDGF- α , $\beta\beta$ and $\alpha\beta$ isomers), transforming growth factor (TGF- β , $\beta1$ and $\beta2$ isomers), platelet factor 4 (PF4), interleukin-1 (IL-1), platelet-derived angiogenesis factor (PDAF), VEGF, epidermal growth factor (EGF), platelet-derived endothelial growth factor (PDEGF), epithelial cell growth factor (ECGF), insulin-like growth factor (IGF), osteocalcin, osteonectin, fibrinogen, vitronectin, fibronectin, and thrombospondin⁵⁰⁻⁵⁶. Adult mesenchymal stem cells, osteoblasts, fibroblasts, endothelial cells and epidermal cells all respond to the abovementioned proteins^{54,57}, thus promoting tissue/bone formation.

BMAC seeded on bone allograft is effective in locally delivering platelet-derived growth factors and MSCs. Nonetheless, before recommending this technique to treat bone defects resulting from aseptic loosening of acetabular cups in THAs, we believe that further research is required. We encourage performing large, multicenter, prospective, controlled studies with a long follow-up in order to draw more conclusive results.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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