

# Comparative Study on the Application of Mesenchymal Stromal Cells Combined with Tricalcium Phosphate Scaffold into Femoral Bone Defects

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## Abstract

This prospective study sought to evaluate the healing quality of implanted ultraporous  $\beta$ -tricalcium phosphate sown with expanded autologous mesenchymal stromal cells (MSCs) into femoral defects during revision hip arthroplasty. A total of 37 osseous defects in 37 patients were treated and evaluated concerning bone regeneration. Nineteen subjects received  $\beta$ -tricalcium phosphate graft material serving as a carrier of expanded autologous MSCs (the trial group A), nine subjects received  $\beta$ -tricalcium phosphate graft material only (the study group B) and nine subjects received cancellous allografts only (the control group C). Clinical and radiographic evaluations were scheduled at 6 weeks, 3, 6, and 12 months post-operatively, and performed at the most recent visit as well. All observed complications were recorded during follow-up to assess the use of an ultraporous  $\beta$ -tricalcium phosphate synthetic graft material combined with expanded MSCs in bone defect repair. The resulting data from participants with accomplished follow-up were processed and statistically evaluated with a Freeman–Halton modification of the Fischer’s exact test, a  $P < 0.05$  value was considered to be significant. Whereas no significant difference was observed between the trial group A with  $\beta$ -tricalcium phosphate synthetic graft material serving as a carrier of expanded autologous MSCs and control group C with cancellous impaction allografting in terms of the bone defect healing, significant differences were documented between the study group B with  $\beta$ -tricalcium phosphate graft material only and control group C. Regarding adverse effects, six serious events were recorded during the clinical trial with no causal relationship to the cell product.  $\beta$ -tricalcium phosphate synthetic graft material serving as a carrier of expanded autologous MSCs appears safe and promotes the healing of bone defects in a jeopardized and/or impaired microenvironment. This clinical trial was registered at the EU Clinical Trials Register before patient recruitment (Registration number: EudraCT number 2012-005599-33; Date of registration: 2013-02-04).

## Keywords

mesenchymal stromal cells, scaffold, bone defect, cell therapy

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## Introduction

Without doubt, the implantation of a total hip arthroplasty is a highly efficient surgical technique leading to pain reduction, restoration of joint function and improvement in the patient's quality of life. With the increasing number of primary total hip joint replacements, together with the treatment of younger and more active patients, a substantial surge in the number of revision operations is to be expected over the next few decades<sup>1,2</sup>.

The goal of revision hip surgery is to create a stable assembly, protect bone and soft tissues, augment bone deficits, recover the biomechanical function of the hip and create the pre-conditions for any future operation. Femur bone defects may result from aseptic loosening, osteolysis, infection, periprosthetic fractures, stress shielding and implant extraction<sup>3,4</sup>. Femur reconstruction during a revision total hip replacement may be the most demanding phase of the operation<sup>5</sup>.

Although local autologous bone grafts are still considered the gold standard in the treatment of minor bone defects, cancellous impaction allografting is a reconstructive surgical option in femoral revision surgery with excellent medium-term clinical and radiological results<sup>6</sup>. Though the clinical benefits of impaction grafting are beyond doubt and the initial steps toward creating an environment for bone regeneration have been taken, cadaver specimens and bone biopsies have shown not only viable trabecular bone, but also composite of graft particles and fibrous tissue, necrotic graft with no vascular invasion, and fibrous tissue membrane within medullary contents<sup>7,8</sup>.

The restoration of large bone defects remains a real challenge<sup>9-11</sup>. Based on our clinical experience, the circumstances of femoral revision dictate a compromised microenvironment with poor vascularization into neighboring implanted metallic endoprosthesis<sup>12</sup>. Recently, regenerative medicine has aimed toward the use of tissue-engineered implants to repair bone defects, showing promising preliminary results in individual cases<sup>13,14</sup>. Although preliminary results are auspicious, the potential clinical indications for stem cell therapy should be further discussed and selected.

In this regard, we designed a prospective, controlled clinical trial using expanded autologous mesenchymal stromal cells (MSCs) for the regeneration of bone defects. This study compares the grade of healing following the implantation of ultraporous  $\beta$ -tricalcium phosphate synthetic graft material (Vitoss<sup>®</sup>, Stryker, Kalamazoo, USA) serving as a carrier for expanded autologous MSCs (suspension of autologous MSC 3P in 1.5 ml; Bioinova, Ltd, Prague, Czech Republic) with the grade of healing following the implantation of  $\beta$ -tricalcium phosphate only and cancellous impaction allografting into femoral defects during revision hip arthroplasty.

## Materials and Methods

### Patients

A total of 177 consecutive revision hip arthroplasties was performed between January 2013 and December 2016.

Following the approval of regulations set by the Local Ethics Committee (authorization confirmed 2013-01-10, identification number 201301/S05 m) and the State Institute for Drug Control of the Czech Republic (authorization confirmed 2013-06-10; identification number 21904/13-I), 37 patients requiring a femoral revision were recruited to participate in this phase IIa trial (protocol AMSC-BDT-001) - EudraCT number 2012-005599-33 (registration 2013-02-04). The participants with an aseptic loosening of a total hip arthroplasty (ASA score I to III for subjects aged 18 to 65 years or ASA score I to II for subjects aged 66 to 76 years, proximal femoral defect seen on plain radiographs) were included if they were able and willing to read, understand, and sign an informed consent form. Patients were excluded from participation if they had isolated acetabular revision, femoral revision without femoral bone loss or one of the following conditions: active infectious disease, previous infection at the site of total hip arthroplasty, cancer, pelvic radionecrosis, rheumatoid arthritis, steroid or immunosuppressive therapy, malnutrition, pregnancy, alcohol or drug abuse, any significant medical condition that would compromise the condition of the patient (congestive heart failure, recent myocardial infarction, kidney or liver failure). The average age of the patients at the time of operation was mean 65.2 (range 44–75) years in the trial group A (11 female; 8 male), mean 67.3 (range 57–75) years in the study group B (female 7; male 2) and mean 71.3 (range 63–76) in the control group C (female 4; male 5) (Table 1).

### Study Design

From January 2013 to December 2016, 37 femoral defects in 37 patients were treated and assessed for bone regeneration. Nineteen subjects received  $\beta$ -tricalcium phosphate graft material serving as a carrier of expanded autologous MSCs (the trial group A), nine subjects received  $\beta$ -tricalcium phosphate graft material only (the study group B) and nine subjects received cancellous allografts only (the control group C). Although surgeon and patient masking was not possible due to the nature of the trial group requiring bone marrow aspiration, the radiographic assessment was done independently by examiners of primary outcome measures. The trial timeline is seen in Fig. 1.

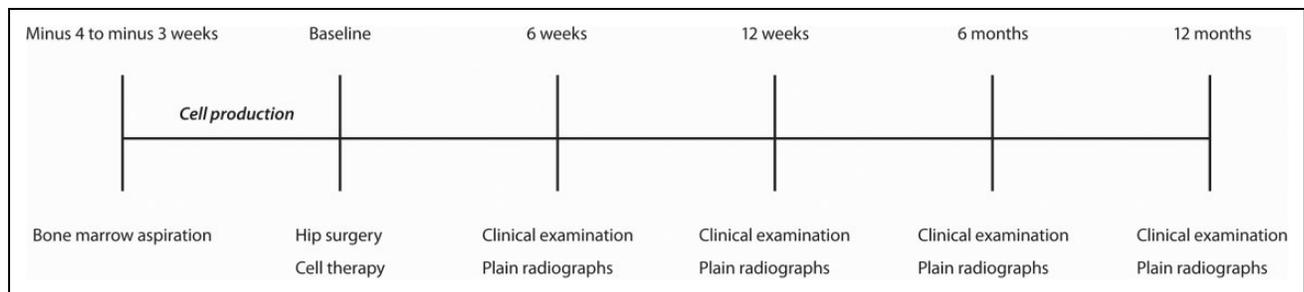
### Clinical Procedures

The production process of MSCs is in accordance with the good manufacturing practice (GMP) guidelines<sup>15</sup>. Following the Local Ethics Committee and the State Institute for Drug Control approval, bone marrow aspiration from anterior ilium under local anesthetic was done in the 19 participants after their informed consent (Fig. 2). The collected bone marrow (10–12 ml) was transported in a sterile collection kit (Bioinova, Ltd., Prague, Czech Republic) to a GMP facility (Bioinova, Ltd.). Afterwards, the bone marrow was

**Table 1.** Demographics of patients.

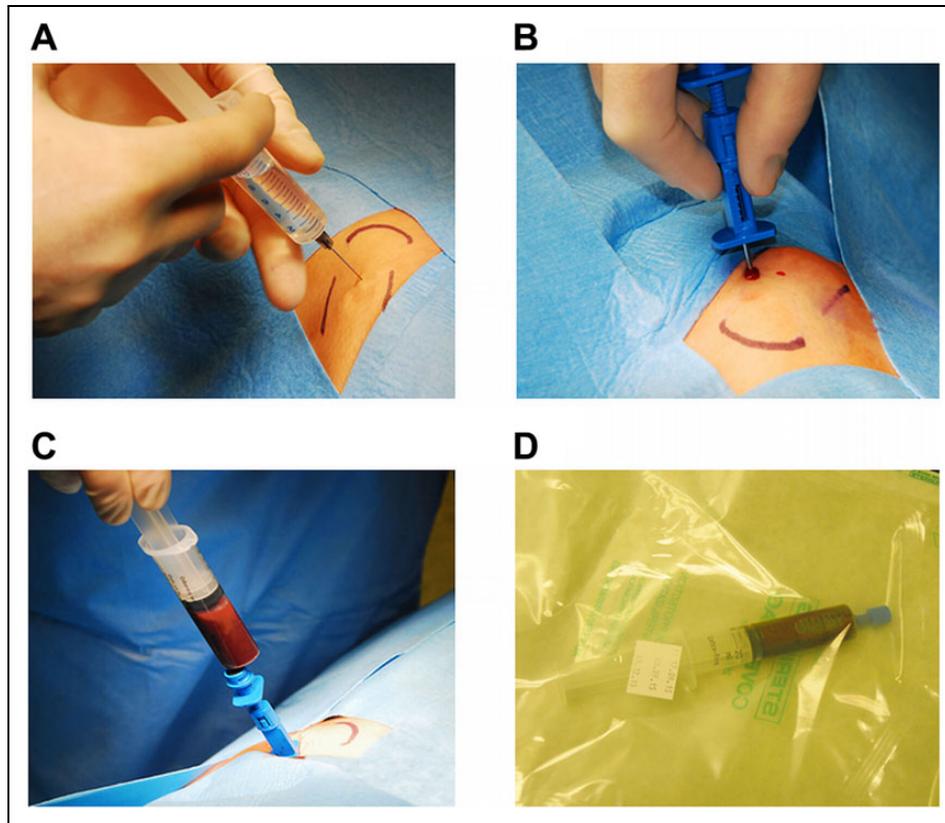
	Trial group (A) (n=19)	Study group (B) (n=9)	Control group (C) (n=9)
<b>Patients</b>			
Age (mean; range):	65.2 (range 44–75)	67.3 (range 57–75)	71.3 (range 63–76)
Weight (mean; range; kg):	78.1 (range 50–106)	77.0 (range 54–94)	77.6 (range 60–93)
BMI (mean; range):	28.2 (range 20–37)	28.6 (range 20–33)	27.3 (range 22–32)
<b>Primary diagnosis</b>			
Osteoarthritis:	6	5	9
Hip dysplasia:	9	4	-
Osteonecrosis:	3	-	-
Femoral neck fracture:	1	-	-
<b>Surgery</b>			
Number of previous revision (mean; range):	0.6 (range 0–3)	0.1 (range 0–1)	1.8 (range 0–7)
Size of the defect (mean; range; cm <sup>3</sup> ):	14.4 (range 4.6–50.0)	15.0 (range 4.5–24.0)	29.0 (range 6.0–52.8)
Revision indication (number of cases):			
Aseptic loosening	18	9	9
Instability of arthroplasty	1	-	-
Cup revision (number of cases):			
None	11	3	3
Uncemented cup	1	1	-
Uncemented cup + morselized all. bone graft (MABG)	6	5	6
Cemented cup + Burch Schneider + MABG	1	-	-
Femoral component removed (number of cases):			
Well fixed	1	2	2
Cemented	14	6	4
Uncemented	4	1	3
AAOS defect classification (number of cases):			
Cavitary	8	9	5
Combined segmental and cavitary	11	-	4
Femoral reinforcement (number of cases):			
None	12	7	8
Cerclage strips (transfemoral approach)	7	2	-
Metal mesh	-	-	1
Femoral component inserted (number of cases):			
Well fixed	1	2	2
Cemented	3	2	6
Revision cemented	-	-	-
Revision uncemented	15	5	1

The table describes data concerning the distribution of patients. A (trial group): patients received beta-tricalcium phosphate graft material serving as a carrier of expanded autologous MSCs. B (study group): patients received  $\beta$ -tricalcium phosphate graft material only. C (control group): patients received cancellous allografts only.

**Figure 1.** Study timeline.

applied on Gelofusine<sup>®</sup> (B. Braun, Melsungen AG, Germany), and the mononuclear fraction was collected and used for cultivation. The cells were sown in plastic flasks (70,000–140,000 cells/cm<sup>2</sup>, TPP Techno Plastic Products

AG, Trasadingen, Switzerland), and the mononuclear fraction was allowed to adhere. Non-adherent cells were removed after 24–48 h by decanting the media. Adherent cells were cultured at 37°C in a humidified atmosphere

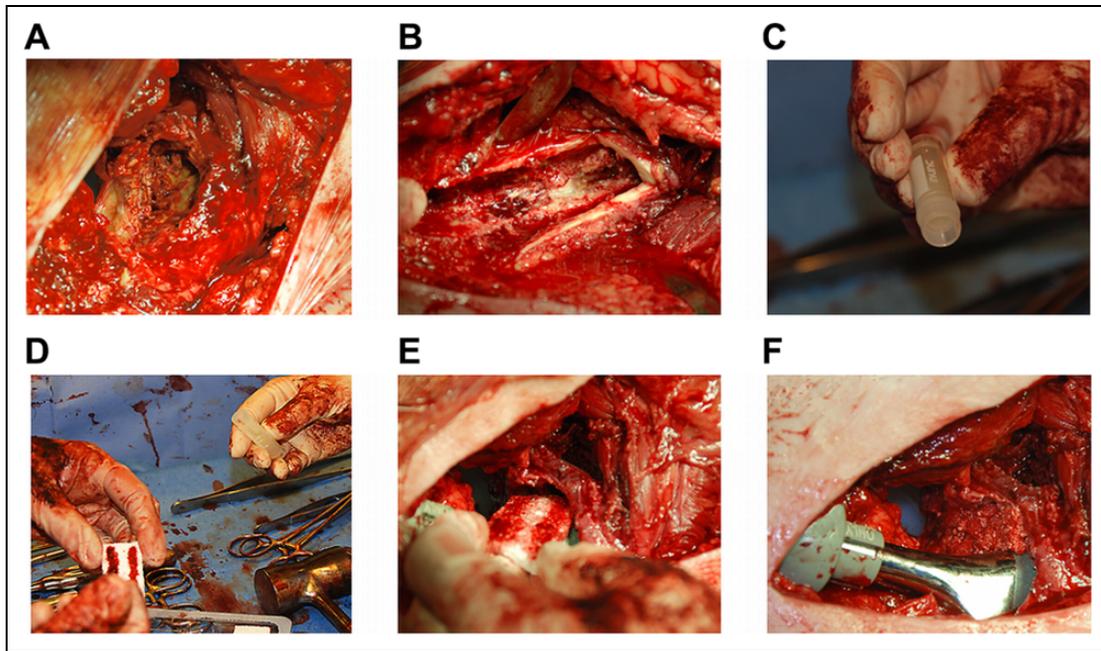


**Figure 2.** A: Local anesthesia in the area of the anterior ilium; B: Insertion of the needle into the bone after skin mini-incision; C: Bone marrow aspiration; D: Collected bone marrow in a sterile collection kit.

containing 5% CO<sub>2</sub> in enriched MEM Alpha (Lonza, Walkersville, Maryland, USA) media, containing platelet lysate (5%; Bioinova, Ltd.) and gentamycin (10 µg/ml; Gentamicine Lek<sup>®</sup>; Lek Pharmaceuticals, Ljubljana, Slovenia); media was replaced twice a week. The cultured cells were identified as MSCs according to their spindle-shaped morphology and plastic adherence. After reaching near-confluency, cells were harvested using TrypLE™ (Life Technologies, Carlsbad, California, USA), and passaged onto a fresh plastic flask. During the third passage (4 weeks after the initial seeding), cells were harvested, counted, and tested for MSC surface markers: CD105/CD73/(CD90/MHC1: 87.8%; SD ± 5.6%; CD16: 1.1%; SD ± 0.8; CD45: 1.2%; SD ± 0.5%; CD34: 1.6%; SD ± 0.7%; CD19: 1.4%; SD ± 0.6; CD3: 1.2%; SD ± 0.4%; CD14: 1.3%; SD ± 0.7%; CD80: 1.3%; SD ± 0.6%; HLA DR (MHCII): 3.1%; SD ± 1.1% and microbiological contamination<sup>16</sup>. The cell product, a suspension of autologous MSC 3P (15 ± 4.5 × 10<sup>6</sup> autologous MSCs/1.5 ml), was then released for individual bone defect treatment. Several product benchmarks were used for MSC multi-lineage potential assessment, testing differentiation into adipogenic, osteogenic and chondrogenic cell lineages.

In all patients participating in this study with the loosening of the previously implanted hip arthroplasty, the

replacement was done with careful curettage of scar and granulation tissue from the prosthetic bed following a Bauer approach. After anchorage of the revision prosthesis into its definitive position, the size of the bone defect was measured. In the trial group A (*n*=19), a volume of 1.5 ml of MSC suspension (15 ± 4.5 × 10<sup>6</sup> cells) was applied onto an absorbable porous β-tricalcium phosphate [β-Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>] sponge (Vitoss<sup>®</sup>, Stryker, Kalamazoo, USA), 5 cc soaked in 3.5 ml autologous blood. The sponge was then inserted into the femoral defect, ensuring adequate contact between implant and margins of bone before wound closure (Fig. 3). The absorbable porous β-tricalcium phosphate [β-Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>] sponge (Vitoss<sup>®</sup>, Stryker, Kalamazoo, USA) only, 5 cc soaked in 3.5 ml autologous blood and 1.5 ml sterile saline, was implanted into the bone defect in the study group B (*n*=9). Fresh-frozen femoral head allografts from our bone bank were used to reconstruct the femur in the control group C (*n*=9). They were morcellized manually into pieces of 0.4–0.6 cm in size and impaction grafting was performed as described by Gie and colleagues<sup>17</sup>. Antibiotic prophylaxis lasted 24 h and low-molecular-weight heparin was used to prevent thromboembolic disease during the following 5 weeks. Post-operative physiotherapy began at the first post-op day, and full weight-bearing was started after 12–24 weeks.



**Figure 3.** A: Proximal femur after removal of the loosened femoral component; B: Bone defect after curettage of the scar and granulation tissue from the prosthetic bed; C: the MSCs suspension ( $15 \pm 4.5 \times 10^6$  cells) in a sterile container; D: the MSCs suspension applied onto an absorbable sponge, 5 cc soaked in 3.5 ml autologous blood; E: Insertion of the cellular absorbable sponge into the femoral defect; F: Completely filled bone defect located medially from the femoral component.

### Outcome Assessments

Clinical and radiographic examinations of all patients were scheduled 1 day before surgery, 6 weeks, and 3, 6, 9 and 12 months, post-operatively. We performed clinical and radiographic examinations at each scheduled time point as well as at the most recent visit to the Department of Orthopaedic Surgery. The Harris hip score was counted to assess pain and function<sup>18</sup>.

The standard radiographs were evaluated independently by three reviewers, and the data were revised for inter-observer agreement. In case of discrepancy, the patient's radiograph was studied by all three observers together. The classification system of the American Academy of Orthopaedic Surgeons was used to assess femoral bone defects<sup>19</sup>. In the final follow-up, the bone defect healing was classified using the Gie guidelines, highlighting the following main observations<sup>17</sup>:

1. No change: radiographic finding of a completely unchanged bone graft material compared with the first post-operative radiograph.
2. Trabecular incorporation: radiographic finding of any change in the structure of the bone graft material from the post-operative radiograph, but without any distinctive orientation.
3. Trabecular remodeling: radiographic finding of the bone graft material changed into a pattern of trabeculae running from the endosteal cortex into the implant along the supposed lines of stress.

4. Cortical healing: radiographic finding of post-operative thickening of the cortex compared with a pre-operatively thinned-out cortex, or a cortex with localized endosteal erosions. The term cortical healing is not applicable when the cortex is not interfered with by the loosening process.

Because cortical healing describes only the reaction in cortical bone, and trabecular incorporation/remodeling relate to the space between the endosteum and implanted endoprosthesis, combined findings were possible. The occurrence of heterotopic ossification was graded using the classification described by Brooker et al<sup>20</sup>.

All complications were recorded during follow-up appointments to assess effect of an ultraporous  $\beta$ -tricalcium phosphate synthetic graft material combined with expanded MSCs in bone defect repair.

### Statistical Analysis

The measurement data were processed and statistically evaluated with the help of MS Excel 2007 (Microsoft Corp, Redmond WA, USA). We used contingency tables for evaluation of possible differences between the corresponding pairs of the tested groups. Since there was low number of observations for some parameters of the tested groups, we opted for using the Freeman–Halton modification of the Fischer's exact test<sup>21</sup>. A value of  $P < 0.05$  was considered to be significant.

**Table 2.** Results of patients.

	Trial group (A) (n=19)*	Study group (B) (n=9)	Control group (C) (n=9)
Follow-up (mean; range; month):	20.7 (range 12–36)	32.0 (range 24–36)	17.3 (range 12–24)
Pre-operative Harris hip score $\pm$ post-operative (mean; points):	48+43	56+30	49+39
Pre-operative pain score $\pm$ the post-operative increase (mean; points):	19+25	23+20	17+27
Trendelenburg sign (number of cases):			
Pre-operative positive and post-operative negative	10	5	4
Pre-operative positive and post-operative positive	7	2	4
Pre-operative negative and post-operative negative	1	2	1
Heterotopic ossification pre-operatively (number of cases):			
None	5	8	3
Grade I	7	1	4
Grade II	–	–	–
Grade III	4	–	1
Grade IV	3	–	1
Heterotopic ossification at latest follow-up (number of cases):			
None	2	7	2
Grade I	15	2	4
Grade II	1	–	1
Grade III	–	–	2
Grade IV	–	–	–
Radiographic result (number of cases):			
No change	–	3	–
Trabecular incorporation	1	5	1
Trabecular remodeling	17	1	8

\* one patient lost to further follow-up 6 months after surgery

The table describes the results of the clinical trial—follow-up, Harris hip score and pain score, Trendelenburg sign, heterotopic ossification pre-operatively (grading), heterotopic ossification at latest follow-up (grading) and radiographic result (evaluation of X-ray).

## Results

### Study Design and Patients

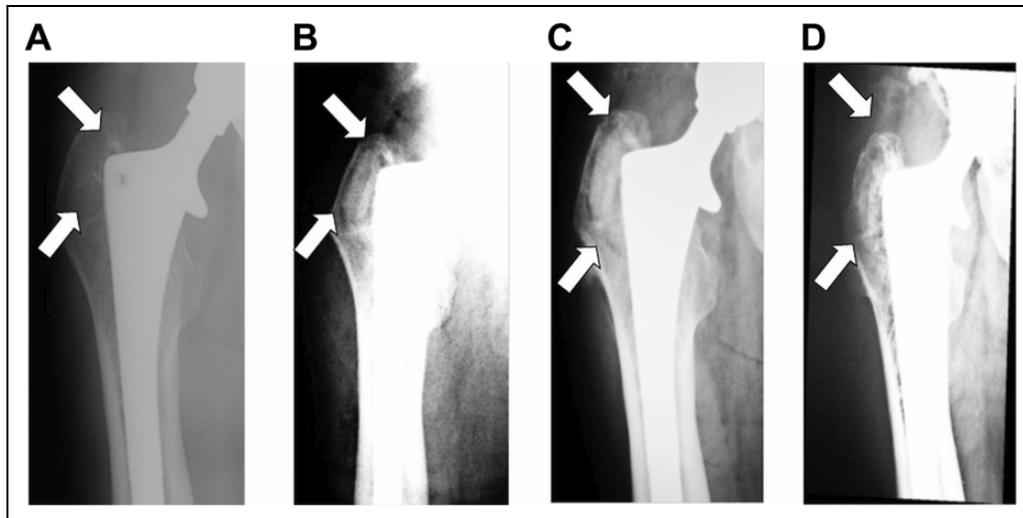
Initially, 20 participants were to be included in the trial group A; however, bone marrow could not be processed from one patient due to aspirate clotting, this patient refused repeated aspiration leaving only 19 patients in the trial group A. The scheduled clinical and radiographic follow-up was accomplished in 36 participants; one participant was lost to follow-up after 6 months.

### Clinical Results

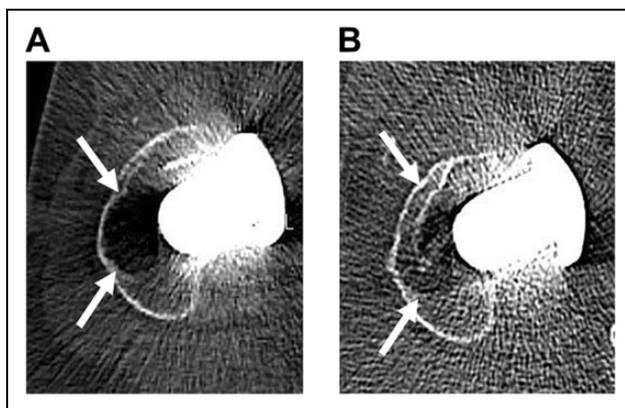
To date, none of the implants has been re-revised. In the trial group A, the mean Harris hip score improved from 48 points to 91 points and the average pain score improved by 25 points to 44 points, post-operatively (Table 2). The mean Harris hip score enhanced from 56 points to 86 points and the average pain score enhanced by 20 points to 43 points in the study group B, whereas in the control group C, the mean Harris hip score improved from 49 points to 88 points and the average pain score improved by 27 points to 44 points, post-operatively (Table 2).

### Radiography

In all 37 patients, radiographs obtained immediately after surgery revealed correct position of the revision prosthesis and adequate filling of the bone defect. In the trial group A with  $\beta$ -tricalcium phosphate synthetic graft material serving as a carrier of expanded autologous MSCs, periodic assessments showed trabecular incorporation of the bone graft material at 6 months after surgery in all cases. Continuing radiographic observations up to 12 months after bone defect treatment have shown trabecular remodeling in 17 of 18 followed patients with distinct bridging bone trabeculae running from the endosteal cortex to the implanted prosthesis. The course of bone defect healing using a plain radiograph is seen in Fig. 4, with a supplementary computed tomography scan in Fig. 5. In addition, two patients had a cortical defect in zone 1 and zone 7, respectively, but both showed cortical repair. None of the implants showed any signs of subsidence. Whereas, over the 12-month follow-up period, no significant difference was observed between the trial group A with  $\beta$ -tricalcium phosphate synthetic graft material serving as a carrier of expanded autologous MSCs, and control group C with cancellous impaction allografting in terms of the bone defect healing, significant differences were documented



**Figure 4.** A: Anteroposterior radiograph of a 61-year-old man (Patient No. 2) with the lateral bone defect of the femur; B: Complete filling of the bone defect with  $\beta$ -tricalcium phosphate synthetic graft material combined with expanded multipotent mesenchymal stromal cells (MSCs) immediately after surgery; C: Graft material visible within the grafted defect at 6 months after surgery; D: Complete resorption of tricalcium phosphate synthetic graft material with trabecular remodeling at 12 months after surgery. All described bone changes are marked by arrows.



**Figure 5.** A: Corresponding transversal CT scan obtained for the same patient shown in previous figures (Patient No. 2) before surgery with cavitory bone defect laterally to the femoral component (marked by arrows); B: Transversal CT scan taken at 12 months after surgery; the defect was filled with tricalcium phosphate scaffold combined with expanded multipotent mesenchymal stromal cells. The femoral defect is completely healed with trabecular remodeling.

between the study group B with  $\beta$ -tricalcium phosphate graft material only and control group C with cancellous impaction allografting ( $P = 0.002$ ) (Table 2).

### Adverse Effects

Serious adverse events recorded during the prospective clinical trial were forwarded to the pharmacovigilance supervisor and to the drug regulatory authority. In the trial

group A, one patient suffered pulmonary embolism 10 weeks following surgery and was successfully treated by thrombolysis. One patient underwent a closed reduction of a correctly implanted prosthesis which dislocated 4 months following the surgery. In one patient, urinary infection occurred 4 months post-operatively and was treated by antibiotics. One patient missed the clinical examination at 9 months post-operatively and his general practitioner informed us of his death due to failed airway management with obturation by pharyngeal hematoma. One woman underwent gynecological intervention for descensus uteri 11 months following orthopedic surgery and one patient underwent contralateral primary hip arthroplasty due to post-dysplastic osteoarthritis 12 months after revision hip surgery. Based on medical records analysis, no causal relationship with the cell graft was identified.

In the study group B, one patient underwent open reduction for a prosthesis dislocated 5 months following surgery. Intraoperative arrhythmia was pharmacologically treated in one patient.

In the control group C, one patient was successfully treated conservatively for femoral diaphyseal stress fracture 17 months after revision hip surgery.

### Discussion

Selecting the appropriate method for femoral reconstruction during revision hip surgery depends on multiple factors including the patient's characteristics, reason for revision, implants requiring removal, previous surgery, soft tissue and bone lesions, and the surgeon's level of experience. An individualized approach is based on the classification system for

femoral defects, of which several have been developed over the years<sup>3,19</sup>. Use of cemented implants is indicated for smaller femoral defects, particularly in elderly patients. Besides the cement-in-cement technique, which calls for minimum bone defects, the impaction grafting method has been used for a long time<sup>7</sup>. Long cemented femoral components are indicated in biologically old patients who are unable to relieve the limb during the post-operative period<sup>22</sup>. It is generally true, however, that cemented implants are not as frequently used during revision operations of femoral component as uncemented implants, which differ in their degree of rotational and vertical stability. Uncemented revision monoblock stems are appropriate during revision surgery with smaller cavitory and segmentary defects. Modular uncemented revision stems differ in the manner by which they achieve vertical stability. The fit and fill principle is based on an exact preparation of the bone-bed in the defective proximal femur and subsequent exact implantation of the proximal module<sup>23</sup>. If the proximal femur is not able to bear the vertical load, a modular tapered fluted stem is indicated<sup>24</sup>. Reconstruction of large segmentary defects of the proximal femur is possible using a solid allograft (applied as an onlay graft or as composite formed by an allograft of the entire proximal femur and a revision modular stem) or morselized allografts impacted into the defective proximal femur ensuring their shape and cohesion with a titanium mesh<sup>4,7</sup>.

As described in the literature, the ideal bone graft substitute for use in revision hip surgery should provide structural stability with new bone formation through osteoconduction, osteoinduction and substitution<sup>25</sup>. Current advances in manufacturing of bone graft substitutes target these properties. Novel metal bone graft substitutes such as porous tantalum exhibit elasticity similar to bone but with greater strength<sup>26</sup>. They are successfully used in acetabular revision and show good bioactivity, biocompatibility and in-growth properties. But when the local situation around the bone defect is jeopardized and/or impaired, as seen in femoral revision, the use of tissue-engineered implants improved the biological potential allowing bone repair, as described in our previous work<sup>15</sup>. Because of need for controlled trials comparing use of bone graft substitutes with established practice, in the present study we compare the healing following the implantation of tricalcium phosphate synthetic graft serving as a carrier for expanded autologous MSCs with tricalcium phosphate only and cancellous impaction allografting into femoral defects during revision hip arthroplasty.

MSCs-containing scaffolds implanted in an orthotopic location as a method of cell therapy could work as an alternative to bone grafts in the treatment of bone defects<sup>27</sup>. A major advantage of such a therapeutic approach is not only the preservation of the original bone stock and shorter surgical time, but also unrestricted availability of bone marrow and higher cellular concentration. Pain prevention in the autograft donor site and decreased associated morbidity should not be omitted too. However, although MSCs show prominent multi-lineage differentiation potential, recent

studies have disclosed that this cellular feature contributes little to their therapeutic benefits<sup>28</sup>. It is widely acknowledged that the therapeutic potential of MSCs is rather derived from their secretion of a variety of growth factors and cytokines<sup>29,30</sup>.

In the present study, no significant difference was observed between the bone defect healing following the implantation of tricalcium phosphate synthetic graft serving as a carrier for expanded autologous MSCs and cancellous impaction allografting into femoral defects, whereas significant differences were documented following the implantation of tricalcium phosphate only and cancellous impaction allografting. No causal relationship to the cell product suspension of autologous MSC 3P was identified in any of the registered serious adverse events during our prospective clinical trial. The serious adverse events recorded emerged rather from the demanding and extensive character of the operation. Both patient and surgeon must be aware of the risks associated with revision hip replacement surgery. The overall complication rate is significantly higher after revision than after primary total hip replacement. The published rate of adverse outcome occurring within 90 days after revision total hip replacement is of 1.24–2.6% for mortality, 0.8–1.08% for pulmonary embolism, 0.95–1.2% for wound infection, 10.0% for hospital re-admission, and 7.4–8.4% for hip dislocation<sup>31–35</sup>. We observed no wound healing complication, hematoma, seroma, surgical site infection or fever. The distribution of heterotopic ossifications corresponded with pre-operative location. There were no tight junctions between the filled defect and heterotopic ossification.

This prospective study is limited by the small number of patients and short follow-up duration. However, because of the complexity involved in preparing and handling living tissue, and the need of absolute coordination between the manufacturing process and surgery, large-scale multicenter applications are not yet possible in the study of tissue-engineered implants and repair of bone defects. In fact, the current literature is deficient in long-term follow-up of larger series and in controlled trials comparing bone graft substitutes with allografts<sup>36</sup>. Nevertheless, the follow-up period in our survey was sufficient to reveal the evident healing of bone defects. Furthermore, the follow-up duration and revision hip replacement complication rate are comparable to those of recent surveys<sup>37,38</sup>. In contrast to animal studies, a biopsy could not be performed as a standard procedure, particularly in uncomplaining patients, due to ethical constraints. The presence of a femoral component and cerclage strips has impeded the use of conventional computed tomography and magnetic resonance imaging because of metal-induced artifacts. Metallic materials in an anatomic area of interest produce both a large signal void area and extensive distortion around the implant, precluding the acquisition of valid information in the immediate area of the metallic object<sup>39</sup>. However, based on histological and radiographic observation, trabecular remodeling and cortical healing have been reported as radiographic changes

concurring with viable bone development<sup>8</sup>. Despite the weakness, our survey is the first prospective controlled trial comparing the use of bone graft substitutes serving as a carrier for expanded autologous MSCs with impaction allografting into femoral bone defects.

Although tissue engineering approaches which utilize the use of stem cells remain a promising clinical approach, further research on stem cell therapy for the repair and regeneration of bone must be focused on clarifying many issues<sup>40</sup>. The ideal stem cell or combination of stem cells for enhancing bone healing must be verified. The mechanisms by which stem cell therapy accentuates bone repair and regeneration need further clarification. Our short-term evaluation of stem cell therapy for the repair of bone defects can help to advance the understanding of the cellular and molecular requirements for effective bone healing.

## Conclusion

In conclusion, our current study demonstrated that  $\beta$ -tricalcium phosphate synthetic graft material serving as a carrier of expanded autologous MSCs may promote the healing in poorly vascularized bone defects neighboring implanted metallic endoprostheses; according to our tests, the first steps toward the origination of a microenvironment for bona fide bone regeneration have been taken.

Our results indicate that the use of autologous MSCs combined with a  $\beta$ -tricalcium phosphate scaffold produced bone regeneration comparable with cancellous impaction allografting when treating femur bone defects with jeopardized and/or impaired microenvironment.

The use of  $\beta$ -tricalcium phosphate synthetic scaffold implants containing expanded MSCs appears to be effective because the serious adverse events recorded during our study emerged from the demanding and extensive character of revision hip replacement without causal relationship to the suspension of autologous MSC. Although the patients improved in all clinical assessments and none of the implants required re-revision, long-term clinical and radiographic follow-up is necessary.

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## Ethical Approval

This study was approved by the Ethics Committee of University Hospital in Hradec Králové (authorization confirmed 2013-01-10, identification number 201301/S05m).

## Statement of Human and Animal Rights

Our prospective, controlled clinical trial was designed to respect the human rights of trial participants according to Helsinki Declaration. This study was approved by the State Institute for Drug

Control of the Czech Republic (authorization confirmed 2013-06-10, identification number 21904/13-1).

## Statement of Informed Consent

An informed consent was signed by patients voluntarily before entering in the study.

## Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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