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BACKGROUND: The authors applied a scaffold-free osteogenic three-dimensional (3D) graft made of adipose-derived mesenchymal stem cells (AMSCs) in patients undergoing minimally invasive transformaminal lumbar interbody fusion (MI-TLIF). METHODS: Three patients (two patients and one patient with 1 and 2 levels, respectively) with degenerative spondylolisthesis underwent MI-TLIF with 3D graft made of AMSCs. To obtain the AMSCs, fatty tissue was collected from the abdomen by lipoaspiration and differentiated afterwards in our Cell/Tissue bank. Clinical outcomes, including the Oswestry Disability Index (ODI) and visual analog scale (VAS) as well as fusion status were assessed preoperatively and up to 12 months postoperatively. RESULTS: At 12 months, all four operated AMSC levels could be assessed (n=4). Grade 3 fusion could be confirmed at two levels out of four. Mean VAS score improved from 8.3 to 2 and ODI also improved from 47 to 31%. No donor site complication was observed. The f...

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Application of a three-dimensional graft of autologous osteodifferentiated adipose stem cells in patients undergoing minimally invasive transforaminal lumbar interbody fusion: clinical proof of concept

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Abstract

Background The authors applied a scaffold-free osteogenic three-dimensional (3D) graft made of adipose-derived mesenchymal stem cells (AMSCs) in patients undergoing minimally invasive transforaminal lumbar interbody fusion (MI-TLIF).

Methods Three patients (two patients and one patient with 1 and 2 levels, respectively) with degenerative spondylolisthesis underwent MI-TLIF with 3D graft made of AMSCs. To obtain the AMSCs, fatty tissue was collected from the abdomen by liposuction and differentiated afterwards in our Cell/Tissue bank. Clinical outcomes, including the Oswestry Disability Index (ODI) and visual analog scale (VAS) as well as fusion status were assessed preoperatively and up to 12 months postoperatively.

Results At 12 months, all four operated AMSC levels could be assessed (n = 4). Grade 3 fusion could be confirmed at two levels out of four. Mean VAS score improved from 8.3 to 2 and ODI also improved from 47 to 31%. No donor site complication was observed. The final AMSC osteogenic product was stable, did not rupture with forceps manipulation, and was easily implanted directly into the cage with no marked modification of operating time.

Conclusions A scaffold-free 3D graft made of AMSCs can be manufactured and used as a promising alternative for spinal fusion procedures. Nevertheless, further studies of a larger series of patients are needed to confirm its effectiveness.

Keywords Adipose-derived mesenchymal stem cells · Bone graft · Spondylolisthesis · Transforaminal lumbar interbody fusion · Fusion rate

Introduction

Spinal fusion procedures are widely used in the treatment of various morbidities, such as deformity, trauma, and degenerative disc disease, associated with instability [17]. Minimally invasive transforaminal lumbar interbody fusion (MI-TLIF) is now one of the most frequently used procedures [37]. Over the last few decades, autologous bone grafting has been used in patients undergoing MI-TLIF [45, 49]. This type of graft is considered the gold standard for spinal fusion, but it is associated with various adverse effects. The harvesting of autologous bone can be associated with persistent donor site pain, paresthesia, hematoma, and infection. Keller et al. and Summers et al. reported major complication rates ranging from 0.76 to 25% [16, 39].

Bone substitutes, such as ceramics, bone morphogenetic proteins (BMPs), and demineralized bone matrix (DBM), have been developed to prevent the problem of donor site morbidity. Ceramics are osteoconductive, biodegradable bone graft scaffolds and should be combined with a local bone graft to enhance their osteoinductive and osteogenic potential [10, 13, 28]. BMP/INFUSE (Medtronic, Memphis, MN, USA), a
potent stimulator of the differentiation of osteoprogenitor cells into osteoblasts, has been used as an iliac crest bone graft substitute in the spine, but many authors have reported complications associated with its application, ranging from 0.66 to 44% [2, 8, 12, 21, 31, 38]. The rate or the nature of the complications differs according to the site of the fusion. Dysphasia and dysphonia have been reported for anterior cervical procedures whereas vertebral osteolysis, graft migration or subsidence, postoperative radiculitis, and heterotropic or ectopic bone formation are more frequent in other spine regions. Other complications like retrograde ejaculations and hematoma formation have also been reported [3, 14, 23, 38, 40]. Therefore, the Food and Drug Administration has issued a safety warning about the use of this product [35]. Derived from human allograft tissue, DBM has repeatedly shown osteoinduction power with fusion rates varying from 82.7 to 92.6% [9, 15, 41].

More recently, a new graft source, adipose-derived mesenchymal stem cells (AMSCs), has emerged and has proven to be more advantageous than autologous iliac crest bone. Zhu et al. showed that adipose tissue is more readily available and that stem cells can be obtained in large quantities [50]. Similarly, Wfyles et al. recently published that the proliferation capacity was increased by fourfold in AMSCs compared with bone marrow BM-MSCs after 20 days in culture [47]. Yang et al. demonstrated that 1 g of abdominal fat tissue could yield up to around 1×10^6 AMSCs [48]. In contrast, Pittenger et al. reported that of 6×10^6 cells aspirated from bone marrow only 0.001 to 0.01% appeared to be stem cells [26]. Schubert et al. reported a shorter differentiation time for AMSCs than for bone marrow-derived mesenchymal stem cells (BM-MSCs) (6.1 ± 2.3 vs. 9.0 ± 1.9 days, p < 0.001), similar immunomodulation capacities at the osteogenic differentiated and undifferentiated status, greater angiogenic properties in vitro and in vivo (19.6 ± 6.8 vessels/0.016 vs. 10.9 ± 4.9 vessels/0.016 mm², p < 0.005), and greater osteogenic capacity (53.3 ± 7.9 cells/0.16 vs. 19.1 ± 17.6 cells/0.16 mm², p < 0.001) [34]. A review by Werner and coauthors highlighted the reliability and effectiveness of AMSCs as an alternative for bone tissue engineering [46].

We developed a graft made of scaffold-free autologous AMSCs differentiated with DBM in a three-dimensional (3D) osteogenic structure [33]. We previously demonstrated the safety and efficacy of this graft in filling a critical-size femoral bone defect in a preclinical pig non-union model at 6 months post-implantation and in extreme clinical cases of bone tumor resection and congenital/acquired bone non-unions up to 48 months post-transplantation [6, 43]. Complete stem cell differentiation in an osteogenic 3D structure improved the efficacy of bone reconstitution by promoting angiogenesis and osteogenesis and made the process safer by reducing the risk of growth factor release [34].

Encouraged by these results, we initiated the present study to apply AMSCs to neurosurgical spine procedures, namely MI-TLIF by using a scaffold-free osteogenic 3D graft (made of AMSCs) in humans. We hypothesized that AMSCs would achieve a good fusion rate in humans and would therefore, be used as an alternative to other spine fusion grafts.

**Methods**

Ethical approval was obtained from the Catholic University of Louvain Ethics Committee (N°B403201111681).

**Patient population**

From March 2012 through January 2014, we prospectively identified 58 patients eligible for TLIF. All patients had chronic low back and/or leg pain resulting from degenerative disc disease or isthmic spondylolisthesis and were refractory to medical treatment. The patients were divided into two groups, MI-TLIF (n=28) or OPEN-TLIF (n=30), based on the neurosurgeon’s preference. MI-TLIF subjects were categorized according to whether or not they agreed to the AMSC protocol, and nine patients consented. Because of a technical problem during AMSC processing (hypercapnia of > 5% CO₂ in the incubator prevented cell growth), only three subjects (two females and one male) were implanted with AMSC during MI-TLIF procedures.

Patients who refused the protocol as well as those who were not implanted because of the technical problem, underwent MI-TLIF with bank bone (BB) in the form of a combination of inactivated bone and DBM provided by the Cell/Tissue bank of our hospital (Center of Tissue and Cell Therapy, University Hospital Saint-Luc, Brussels, Belgium). The OPEN-TLIF subjects were not included in the study (Fig. 1).

**Graft manufacturing**

The tissue-engineered product is made of two major components: (a) allogenic demineralized bone matrix and (b) autologous adipose stem cells.

(a) Human DBM was provided by the musculoskeletal tissue bank and produced from multi-organ human donors. The diaphysis of femoral or tibial bone was cut and grounded into particles of size 200–700 μm for demineralization. DBM was produced by grinding cortical bone from selected human donors (<45 years old). First, human bone tissue was defatted in acetone (99%) bath overnight and then washed for 2 h in demineralized
Decalcification was performed by immersion in 0.6 N HCl for 3 h (20 ml solution per gram of bone) and agitation done at room temperature. The demineralized bone powder was then rinsed with demineralized water for 2 h and the pH measured. If the pH was too acidic, the DBM was buffered with 0.1 M phosphate solution again under agitation. Finally, the DBM was freeze-dried and weighed. The DBM was sterilized with 25 kGy by gamma irradiation at a temperature of −80 °C.

The osteogenic properties of the DBM were assessed on representative batches by: (i) residual calcium concentration after the demineralization process (measured from the calcium contained in a mean of 1.3 g of DBM vs. non-demineralized bone powder from each donor) and (ii) osteoinduction one month after in vivo implantation in the para-vertebral musculature of nude rats (male, 6–8 weeks old) to quantify new bone formation (presence of bone marrow, osteoblast activity, and new bone formation) by histomorphometry (a standard 300 cross-grid for point counting under microphotography at ×10 magnification; four non-overlapping areas per slide were studied) for demineralized vs. non-demineralized bone matrix.

(b) Autologous adipose stem cells were prepared as described by a cell bank [6, 43]. The Endocrine Cell Therapy Unit is recognized by the Belgian Federal Agency for Medicines and Health Products as a clinical laboratory for the processing of AMSCs. The AMSC expansion and differentiation were performed in line with good manufacturing practices (GMPs) and the ISO 9001–2008 quality management system. All AMSC isolation and expansion procedures were performed in grade A air-laminated flow located in a grade B clean room (validated annually by the ICCE SA, Elsene, Belgium) in accordance with Belgian Ministry...
of Health recommendations and European directives (regulation 1394/2007 for advanced cell therapy products). The environment for cell culture was checked by weekly particle counting (under static and dynamic conditions; Laser II Particle Counter, Particle Measuring Systems Germany GmbH, Darmstadt, Germany) and microbiological testing at each manipulation, as recorded in the “Graft Report”.

The final three-dimensional graft was obtained by multiple steps including:

1. **The adipose tissue digestion and AMSC isolation.**

   For the isolation of human AMSCs, a mean of 4.4 g (range, 3.2–6.5 g) of fatty tissue was harvested under local anesthesia by liposuction (following the Coleman technique in the abdominal region) from patients after informed consent and serologic screening. The adipose tissue was digested with GMP collagenase 0.075 g; 8000 PZ U/L; Serva Electrophoresis GmbH, Heidelberg, Germany. After sequential trypsinizations in a 75-cm² culture flask, AMSCs were then isolated and expanded in the proliferation media (Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated and viral-tested fetal bovine serum certified by the U.S. Department of Agriculture; Life Technologies, Grand Island, NY, USA) up to passage 5 (P5) within 50 ± 6 days to assess their properties in terms of in vitro mesenchymal differentiation capacity (adipogenesis, chondrogenesis, and osteogenesis) and surface markers (CD44, CD45, CD73, CD90, CD105) (see below).

2. **The AMSC differentiation and constitution of the three-dimensional graft.**

   At P5, AMSCs were incubated in 150-cm² culture flasks in osteogenic media composed of proliferation media supplemented with dexamethasone (1 μM), sodium ascorbate (50 μg/ml), and sodium dihydrophosphate (36 mg/ml). After 11 ± 0.6 days of incubation, DBM (10 mg/ml) was added to create a multi-dimensional structure that was ready to use for implantation straight from the plastic dish. The 3D graft was implanted after a mean of 40 ± 14 days of incubation in osteogenic media (after P5).

   The 3D graft was rinsed three times with transplantation media (CMRL; Mediatec Inc., Manassas, VA, USA) without phenol red and without antibiotics or sera. The graft was finally placed in a sterile culture flask enclosed in three sterile plastic bags. The graft was then transferred at room temperature, in less than 15 min, to the operating room for implantation.

   AMSCs (at P5) were tested in specific media to assess the capacity of differentiation towards the three main mesenchymal lineages. Confluent AMSCs cultures were induced to undergo osteogenesis by replacement of the proliferation medium with specific induction media for osteogenesis: proliferation medium supplemented with dexamethasone (1 μM), sodium ascorbate (50 μg/ml), and sodium dihydrophosphate (36 mg/ml). The medium was replaced every 2 days until differentiation could be demonstrated by alizarin red (for CaPO4 deposition) and osteocalcin (for bone phenotype).

3. **The quality control testing of the cell therapy product.**

   A 20-mm² biopsy (on the day of transplantation) was fixed in 4% paraformaldehyde overnight. We normalized the integrity of the 3D graft by the (DBM – ECM)/viable cell ratio (see above) between −1 and +1.

   Cytogenetic stability was studied by karyotype and fluorescence in situ hybridization (FISH) analyses at P4 of the AMSCs (undifferentiated and differentiated) from the three patients, whose procedures were completed without technical problems, to assess the oncogenic safety of the cellular components of the 3D graft. Metaphase chromosomes were obtained according to standard protocols from cultured AMSCs in the exponential growth phase after P4. Twenty Giemsa-Trypsin-Wright-banded metaphases were analyzed and karyotypes were reported according to the 2013 International System for Human Cytogenetics Nomenclature.

   A FISH experiment was performed using the P4 AMSCs (undifferentiated and differentiated) according to standard protocols to detect aneuploidy of chromosomes 7 and 8 using CEP7/D7Z1 (SpectrumGreen or SpectrumOrange) or CEP8/D8Z2 (SpectrumOrange or SpectrumGreen) probes (Abbott Molecular, Ottignies-Louvain-la-Neuve, Belgium) [7]. At least 100 nuclei were counted, and the thresholds were calculated following the inverse beta law with a confidence interval of 99.9%.

   Mycoplasma and endotoxin assays were also performed according to current GMP guidelines using TEXCELL SA (Evry, France) on cellular samples collected at P4 for undifferentiated and osteogenic cells (the last sample prior to graft delivery). Microbiological testing using BACTEC assays was performed at each media change (twice a week during the manufacture of the graft) for aerobic, anaerobic, moisture, and yeast culture.

   In-process controls (on cellular samples collected at P5 for undifferentiated and osteogenic cells up to the last sample prior to graft delivery) based on safety tests showed no microbiological or mycoplasmic contamination and no endotoxin content in any manufactured batch. Thus, all manufactured 3D grafts fulfilled the release criteria for implantation.

4. **The characterization of the osteogenic three-dimensional graft.**
To assess the volumetric bone mineral density of the final 3D graft, peripheral quantitative computed tomography (pQCT, XCT Research SA, Sratec Pforzheim, Germany) and X-ray microtomography (Skyscan 1172 high-resolution desktop XCMT system; Skyscan, Aartselaar, Belgium) were performed on each sample. Two samples were analyzed by SEM at magnification ×50 and ×300 to analyze the micro-architectural structure of the 3D graft. Interconnections between cells, ECM and DBM were observed and correlate to histomorphometrical analysis. Samples were fixed in glutaraldehyde 2.5% and gradually dehydrated in an ethanol solution from 10 to 100%. Samples were then gold coated before observation.

Surgical procedure

Three months after AMSC graft manufacture, MI-TLIF was performed under general anesthesia. The patient was positioned prone on a radiolucent operating table. Localization and memorization of the vertebral segment and each targeted pedicle was performed by fluoroscopic Zeego 2D guidance (Siemens, Forchheim, Bavaria, Germany). An incision of 2 cm was made 4–5 cm from the midline on the side where the most severe radicular compression was present. A tubular retractor (Pipeline; DePuy Spine, Johnson & Johnson, Arlington, USA) was docked to expose the targeted facet joint. After maximized discectomy, the disc space was filled with AMSC graft and a Concorde cage (DePuy Spine, Johnson & Johnson, Arlington, TX, USA) filled with AMSCs was inserted into the disc space. Fluoroscopic Zeego 3D images were acquired to check for correct cage placement. Guide wires were then placed percutaneously into the pedicles under fluoroscopic Zeego guidance. Percutaneous pedicle screws (PPS) were inserted using the Viper 2 fixation system (DePuy Spine, Johnson & Johnson) and a Zeego 3D sequence confirmed their position. Rods were then slid into the screw heads and tightened. A para-midline “mirror image” incision was made on the contralateral side centered over the targeted pedicles. PPS and rods were connected by the same percutaneous system as that on the TLIF side [27].

Clinical evaluation

Clinical outcomes were examined pre- and post-operatively and during follow-up visits using back and leg pain visual analogue scales (VAS) and the Oswestry Disability Index (ODI).

Radiological evaluation of fusion

Multi-detector spiral CT images were obtained at 6 and 12 months after surgery in all patients implanted with AMSCs. A senior independent radiologist specializing in musculoskeletal radiology analyzed all images on a workstation using the multiplanar software from our Picture Archiving and Communication System. The fusion status of the relevant intervertebral disc was graded: grade 3 (solid fusion) was defined as the formation of a continuous bone bridge across the intervertebral space through or around the cage; grade 2B was described as new trabecular bone extending from the end plates into the disc but without forming a continuous bone bridge; grade 2A was determined as relative prominence of the vertebral end plates due to subtle migration of the interbody space within the bony end plates; and grade 1 was given when there was no evidence of trabecular bone formation extending from the end plates [20].

For the safety evaluation, the radiologist was asked to note any bone formation beyond the interbody cage and any bone resorption from the vertebral end plates.

Statistical analysis

Because of the small sample size, it seemed more statistically appropriate to use descriptive than inferential statistics. We accordingly report proportions of the interbody level disc fused, means (age, pain duration, VAS and ODI), and standard deviations. No statistical tests were performed.

Results

Graft characteristics

At P4, the characteristics of the human AMSCs were confirmed by differentiation into adipose, osteogenic, and chondrogenic phenotypes and by a significant shift in the mean fluorescence intensity curve (FACS) for CD44 (>99.9%), CD73 (>96%), CD90 (>98%), and CD105 (>97%). CD45 antigen expression was negative (<6%). The quality of the human DBM was confirmed by significant reduction in calcium content (by a mean demineralization of 98%, \( p < 0.005 \)) and by significantly higher in vivo osteogenesis (+11% of the explanted graft with osteoinductivity in representative DBM batches, \( p < 0.05 \)) compared to non-demineralized cortical bone matrix.

A mean of 20 ± 4 million AMSCs per patient was available by the end of P4 and were sufficient for seeding into three culture flasks of 150 cm² for P5. Osteogenic differentiation was then induced at P5 for 15 days (when AMSCs were confluent) before supplementation with DBM at 10 mg/ml to create the 3D structure. All grafts showed a 3D structure prior to implantation. The 3D graft was implanted 89 ± 9 days after adipose tissue procurement. Two to three grafts of 3×3 cm² (one graft per 150 cm² flask) per patient were
produced from AMSCs supplemented with 10 mg/ml DBM. The final product was stable and did not rupture with forceps manipulation (Fig. 2B3). The integrity of the 3D graft was assessed at the end of manufacturing by the histomorphometric potency score. A score between −1 and +1 (in terms of cellular content, interconnective tissue integrity, and DBM content) was obtained for optimal 3D graft integrity (Fig. 2a).

According to pQCT, a significant degree of mineralization was observed for ASCs incubated with DBM. The optimal concentration of DBM was adjusted with the anticipated function of the 3D construction to produce a sufficiently stable graft for manipulation with forceps and integration in a lumbar cage (Fig 2B1). The 3D structure of ASCs incubated with DBM, which was observed macroscopically, was confirmed by X-ray microtomography (Fig. 2B2). The characterization of the ultrastructure of the 3D graft, by scanning electron microscopy, demonstrated that the interconnective tissue (synthesized by adipose stem cells) promotes the connection between DBM particles (Fig. 2c).

No complex numerical or structural clonal chromosomal aberrations were detected in the AMSCs developed for each graft at P1 and P4 (in both undifferentiated and differentiated status). Minor clones with structural aberrations detected in the undifferentiated AMSCs at P4 were absent from the differentiated AMSCs. Minority tetrasomies of both probes by FISH, suggesting tetraploidy, were found for undifferentiated AMSCs. Initial trisomy 8 was not detected, but minority tetraploidy (detected by the tetrasomies of both probes by FISH) was found after osteogenic differentiation.

![Fig. 2](image-url)

**Fig. 2** a The graft integrity (for the optimal 3D graft) was confirmed by an hematoxylin-eosin staining (left) with the integrity of the interconnective tissue (extracellular collagen matrix synthesized by adipose stem cells) between DBM (*) as shown by Masson’s trichrome (middle). The cellular viability of adipose stem cells inside the interconnective tissue was confirmed by DAPI staining (right). b The mineralization was confirmed pQCT (B1, white spot for calcium deposition). The 3D structure was confirmed by XCMT (B2). The final 3D structure is shown (B3). c SEM demonstrated that DBM particles are linked by the interconnective tissue (C1) synthesized by adipose stem cells (surrounding DBM particles, C2).
Participants

Three patients received AMSCs. The average age of the population was 48.7 years, with an average pain duration of 2.3 years (Table 1).

No complications associated with surgery were reported. All of the cages were implanted successfully. After surgery, none of the patients showed neurological deterioration. All patients treated with AMSCs were followed up for at least 12 months.

Clinical and radiographic outcomes

The mean VAS score improved from a preoperative value of 8.3 ± 0.5 to 2 ± 1.4 postoperatively. The mean preoperative ODI score was 47 ± 23 and decreased to 31 ± 5 12 months postoperatively.

In total, four levels were implanted with AMSCs. Single-level implantation was performed in 67% (2/3) of the patients. The last patient had two levels implanted (Table 1). At 6 months post-surgery, fusion could be assessed with CT scan in two patients. One patient (with one operated level) had standard X-ray instead of CT scan imaging and therefore, fusion characteristics could not be evaluated adequately. Two out of the three levels evaluated at this time period showed characteristics of solid fusion. At 12 months, two assessed levels demonstrated grade 3 fusion (Table 2). On CT imaging, no adverse local effects, such as bone

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient demographic data</th>
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<tr>
<td><strong>Gender</strong></td>
<td><strong>Male</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Female</strong></td>
</tr>
<tr>
<td><strong>Age (years) ± SD (range)</strong></td>
<td>48.7 ± 14.3 (32–67)</td>
</tr>
<tr>
<td><strong>Mean pain duration (years, range)</strong></td>
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</table>

<1 | 0 |
1-2 | 2 |
>2 | 1 |

<table>
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<tr>
<th><strong>TLIF level</strong></th>
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<td>2*</td>
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<tr>
<td><strong>Two</strong></td>
<td>1*</td>
</tr>
<tr>
<td>L4-L5</td>
<td>2 (50)</td>
</tr>
<tr>
<td>L5-S1</td>
<td>2 (50)</td>
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<table>
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<th><strong>Diagnosis</strong></th>
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</tr>
<tr>
<td>Degenerative spondylolisthesis</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td><strong>DDD</strong></td>
<td>1 (33.3)</td>
</tr>
</tbody>
</table>

**AMSCs** adipose-derived mesenchymal stem cells, **m** months, **BB bank bone**

All figures in parentheses are percentages if not indicated otherwise

**Table 2** Fusion assessment at the operated levels

<table>
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<tr>
<th>No. of patients</th>
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<tr>
<td><strong>Total no. of levels assessed (6 m and 12 m)</strong></td>
<td>7</td>
</tr>
<tr>
<td><strong>No. of levels assessed at 6 months</strong></td>
<td>3</td>
</tr>
<tr>
<td><strong>Fusion grades (%)</strong></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1 (33)</td>
</tr>
<tr>
<td>IIa</td>
<td>0</td>
</tr>
<tr>
<td>IIb</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>2 (67)</td>
</tr>
<tr>
<td><strong>No. of levels assessed at 12 months</strong></td>
<td>4</td>
</tr>
<tr>
<td><strong>Fusion grades (%)</strong></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0</td>
</tr>
<tr>
<td>IIa</td>
<td>0</td>
</tr>
<tr>
<td>IIb</td>
<td>2 (50)</td>
</tr>
<tr>
<td>III</td>
<td>2 (50)</td>
</tr>
</tbody>
</table>

**DDD** degenerative disk disease, **BB bank bone**

* The figure refers to the number of patients undergoing TLIF in single or two level

**SD** standard deviation, **TLIF** transforaminal lumbar interbody fusion

All figures in parenthesis are percentages if not indicated otherwise

**Fig. 3** Sagittal CT scan comparing bridging bone of a patient treated with AMSCs

Discussion

The objective of the present study was to preliminary apply scaffold-free osteogenic 3D grafts from stem cells from abdominal fat tissue in human spine during interbody fusion and stabilization procedures. As said earlier, we previously demonstrated the safety and efficacy of this graft in filling a critical-size femoral bone defect animal model at 6 months post-implantation and in extreme clinical cases of bone tumor resection and congenital/acquired bone non-unions up to 48 months post-transplantation [43]. Our results show that the procedure is applicable in human spine without
complications. There were no donor site complications and the amount of fat tissue that could be collected was unlimited. Fischgrund et al. and Vaccaro et al. reported morbidity associated with the use of autologous iliac crest, including non-union, in up to 55% of cases [11, 42]. Iliac crest autogenous bone grafting is associated with pain, paresthesia, hematoma, and even infection at the donor site in more than 60% of cases [4, 5]. None of our patients showed any of these symptoms after abdominal fat collection, and no complications were observed near the implantation site on post-operative CT imaging.

Mean VAS and ODI scores were improved in all patients at 12 months compared with preoperative values.

There was a period of 30 days between the decision that surgery was indicated and abdominal fat cell harvesting, and implantation was performed 89 days later. Thus, the whole process took 119 days, in contrast to 111 days for patients receiving bank bone. The duration of this new process should not, therefore, be considered a barrier to using stem cells from abdominal fat, especially since the period between the decision to undergo surgery and harvesting could be reduced. Currently, the improvement of the manufacturing significantly reduced the time to obtain the final three-dimensional scaffold free-graft (from adipose stem cells after the native adipose tissue procurement) by 1.5 months.

Another important issue remains the risk of oncogenicity following the use of growth factors and stem cells to promote osteogenesis. BMP-2, the main growth factors contained in DBM and used to control important features of stem cells osteoblastic differentiation through WNT signaling-activating ligands, demonstrated controversial effects on osteogenic differentiation and tumor growth. Luo et al. [19] reported that BMP-2 failed to induce bone formation and instead efficiently promoted tumor growth, while Wang et al. [44] conversely showed that BMP-2 treatment induced the upregulation of terminal osteogenic markers inhibiting their tumorigenic potential. Recently, Rubio et al. [30] confirmed that the in vivo osteoblastic sarcoma developed by a synergistic effect of 40 mg of calcium substrates (hydroxyapatite and tricalciumphosphate) and 35 μg of BMP-2 on 1 × 106 cells human MSCs. In our study, the final product was characterized by a significantly lower concentration of BMP-2 (after protein extraction a mean of 54 ± 13 ng of BMP-2/g of DBM in comparison to 5.5 ± 13.4 ng/g of tissue-engineered product (from adipose stem cells)) in comparison to those reported by Rubio et al. [30]. Indeed, one 3D graft is constituted by the addition of 10 mg/ml of DBM (170 mg of DBM reconstituted in 17 ml of osteogenic media in a 150-cm² flask corresponding to 5.8 ng of BMP-2 since 34.4 ng of BMP-2 is extracted per 1 g of DBM) to a mean of 5.8 × 106 AMSCs (number of cells per flask of 150 cm² exposed to 170 mg of DBM at P4). It was also noted that the concentration of BMP-2 per gram of tissue was significantly reduced (by 85.2%) by the in vitro osteogenic maturation of AMSCs (in combination of DBM in the final 3D graft) in comparison to the equivalent amount of DBM. Although human MSC (deficient for p53 and/or Rb) failed to induce tumor formation in vivo, suggesting the safety of these cells in clinical application, Perrot et al. [25] postulated a risk associated with autologous fat graft implantation in a post-neoplastic context, especially for osteosarcoma. Controversy exists concerning the potential for spontaneous transformation of MSCs after prolonged ex vivo culture, but several studies reported that MSCs have limited tendencies to develop tumors [1, 22, 29]. Our results could indicate (i) the absence of adverse events in patients up to 4 years after implantation for the first implanted patient; (ii) AMSCs delivery after a shorter in vitro culture (P4), thus avoiding the selection of tumor cell clones; (iii) the stabilization of the genome by osteogenic differentiation and (iv) the reduction of the concentration of BMP-2 in the final graft in comparison to DBM alone. But of course, the present series is limited and should be considered as a “proof of concept”.

Another objective of this study was to assess the quality of the fusion using AMSCs. Our results show that this alternative source can achieve lumbar interbody fusion in humans (Fig. 3). The apparent reduction in the fusion rate observed over time (at 12 months compared to 6 months) was due to a difference in the number of levels assessed during the two periods (Table 2), which could lead to this variation in fusion rate in a small population. Our results were analyzed by a blinded independent radiologist who distinguished between grades 3 and 2B.

We are unaware of any other studies using AMSCs in TLIF surgical procedures in humans. Studies of TLIF procedures using local bone grafts have found fusion rates ranging from 76 to 93% [18, 24, 32, 36] in a follow-up evaluation period extending from 6 to 24 months. Larger series are needed to confirm that the use of AMSCs in interbody fusion is not inferior to the use of local bone graft.

From these early results in this limited group of patients, AMSCs appear to provide an attractive alternative to iliac crest bone graft, providing a safe source of stem cells and avoiding the morbidity associated with autologous bone graft harvest.

Conclusions

The implantation of stem cells derived from adipose tissue in human spine has never been reported in the literature. In this preliminary study, the use of AMSCs was reproducible and associated with no major complications. This initial experience represents a promising alternative to current graft materials and needs to be confirmed in future and extensive investigations.
AMSCs, adipose-derived mesenchymal stem cells; BB, bank bone (bone + demineralized bone matrix); BM-MSCs, bone marrow-derived mesenchymal stem cells, BMPs, bone morphogenetic proteins; CT, computed tomography; DBM, demineralized bone matrix; GMP, good manufacturing practice; MI-TLIF, minimally invasive transforaminal lumbar interbody fusion; ODI, Oswestry disability index; VAS, visual analog scale.

Compliance with ethical standards

Funding No funding was received for this research.

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Comments

This is an interesting and important study. It demonstrates that we can already obtain autologous scaffold-free osteogenic grafts from stem cells of fat tissue. The study also demonstrates that we can do it while avoiding any harvesting site complications and without limitation. Nevertheless, I believe this study, at this present stage, remains a proof-of-concept. There are still issues that need to be clarified before we can consider this technique as a definitive alternative for spinal fusion. Even though the authors, in a previous study (1), reported that no complications occurred in a series of 17 patients during short-term or long-term periods after the implantation of the product, questions concerning the genetic safety of cell therapy need further studies to be definitively answered. Furthermore, a multicentric study, involving many patients will be necessary to assess the clinical value of such stem cells-derived autografts as compared with conventional allograft techniques.


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