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The enhanced performance of bone allografts using osteogenic-differentiated adipose-derived mesenchymal stem cells

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ABSTRACT

Adipose tissue was only recently considered as a potential source of mesenchymal stem cells (MSCs) for bone tissue engineering. To improve the osteogenicity of acellular bone allografts, adipose MSCs (AMSCs) and bone marrow MSCs (BM-MSCs) at nondifferentiated and osteogenic-differentiated stages were investigated in vitro and in vivo. In vitro experiments demonstrated a superiority of AMSCs for proliferation (6.1 ± 2.3 days vs. 9.0 ± 1.9 days between each passage for BM-MSCs, respectively, $P < 0.001$). A significantly higher T-cell depletion (revealed by mixed lymphocyte reaction, [MLR]) was found for AMSCs (vs. BM-MSCs) at both non- and differentiated stages. Although nondifferentiated AMSCs secreted a higher amount of vascular endothelial growth factor [VEGF] in vitro (between 24 and 72 h of incubation at 0.1–21% O_2) than BM-MSCs ($P < 0.001$), the osteogenic differentiation induced a significantly higher VEGF release by BM-MSCs at each condition ($P < 0.001$). After implantation in the paraspinal muscles of nude rats, a significantly higher angiogenesis (histomorphometry for vessel development ($P < 0.005$) and VEGF expression ($P < 0.001$)) and osteogenesis (as revealed by osteocalcin expression ($P < 0.001$) and micro-CT imagery for newly formed bone tissue ($P < 0.05$)) were found for osteogenic-differentiated AMSCs in comparison to BM-MSCs after 30 days of implantation. Osteogenic-differentiated AMSCs are the best candidate to improve the angio-/osteogenicity of decellularized bone allografts.

1. Introduction

Massive bone defects occurring after cancer resections, infections, or trauma often require the use of human allografts. The non-consolidation of acellular bone matrix is associated with a lack of osteoinductivity and angiogenic stimulation [1,2]. Many technologies have been designed to improve bone allograft recolonization and to induce tissue revitalization [3–6]. A composite graft made of a bone allograft loaded with mesenchymal stem cells (MSCs) may be proposed as a solution to release growth factors to improve angiogenesis and osteoinductivity.

Bone marrow (BM) has long been considered the main source of MSCs [7,8]. However, difficulties with obtaining sufficient quantities of grafting material, avoiding harvesting-related morbidities, and lacking in vivo reproducibility (in terms of osteogenesis) stimulated the search for alternate sources of MSCs [9].

Recent reports indicated that MSCs can be found in numerous other tissues, which can be harvested and processed more easily than BM. In this context, adipose tissue constitutes a readily accessible, abundant, and easily replenishable source of MSCs [10–13]. Theoretical advantages for bone tissue engineering using adipose mesenchymal stem cells (AMSCs) compared to BM-MSCs include use of a less invasive method for tissue procurement and the ability to obtain greater numbers of stem cells isolated from the original tissue [14]. However, the majority of studies focused on in vitro or in vivo properties of nondifferentiated MSCs, individually for BM or adipose origin, for bone reconstruction.

The present work was designed to compare AMSCs and BM-MSCs, at both nondifferentiated and differentiated (osteogenic) stages for (1) in vitro proliferation and osteogenic differentiation capacities, angiogenic properties by quantifying the release of vascular endothelial growth factor (VEGF) and their immunomodulation

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properties; and (2) in vivo by implanting cancellous bone chips loaded with MSCs (AMSCs/BM-MSCs) to assess osteogenesis and angiogenesis by immunohistochemistry and micro-computed tomography (micro-CT) techniques.

2. Materials and methods

All materials were obtained from Lonza (Basel, Switzerland), Sigma–Aldrich (St. Louis, MO, USA), or Invitrogen (Carlsbad, CA, USA) unless otherwise specified.

2.1. Animal sources of BM-MSCs and AMSCs

Pigs (<100 kg, <6-month-old) used as donors for BM-MSCs and AMSCs were either green fluorescent transgenic pigs, provided by Prof. C. Galli (Cremona, Italy) [15], or Belgian Landrace pigs. Animals were housed according to the guidelines of the Belgian Ministry of Agriculture and Animal Care. All procedures were approved by the local Ethics Committee for Animal Care of the Université catholique de Louvain (Fig. 1).

2.2. Isolation of BM-MSCs and AMSCs

2.2.1. BM-MSCs

Heparinized BM was mixed with a double volume of phosphate-buffered saline (PBS) and centrifuged at 1500 rpm for 10 min. Cells were then resuspended as 10^7 cells/mL and the cell suspension was layered over a Ficoll-Hypaque column (density: 1.077; Lymphoprep, Nycomed, Oslo, Norway) and centrifuged for 30 min at 2500 rpm. The mononuclear cells were collected from the interface and transferred to a fresh tube and washed in PBS at 1500 rpm for 10 min. Cells were resuspended in MSC medium consisting of Dulbecco modified Eagle medium (DMEM), supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin 100 U/mL, and streptomycin 100 μg/mL and placed in culture flasks [16,17].

2.2.2. AMSCs

AMSCs were harvested using a standard protocol as described previously by Mitchell et al. and Cui et al. [18,19]. Collagenase (0.075 g) was reconstituted in Hanks balanced salt solution (with calcium and magnesium ions) and stored at 2–8 °C before digestion. Fatty tissues (a mean of 15 g) were washed three times with 9‰ NaCl and cut in a Petri dish to remove vessels and fibrous connective tissue. The fat was weighed before digestion and transferred in a 50-mL Flacon tube containing the enzyme. The tissue was placed in a shaking water bath at 37 °C with continuous

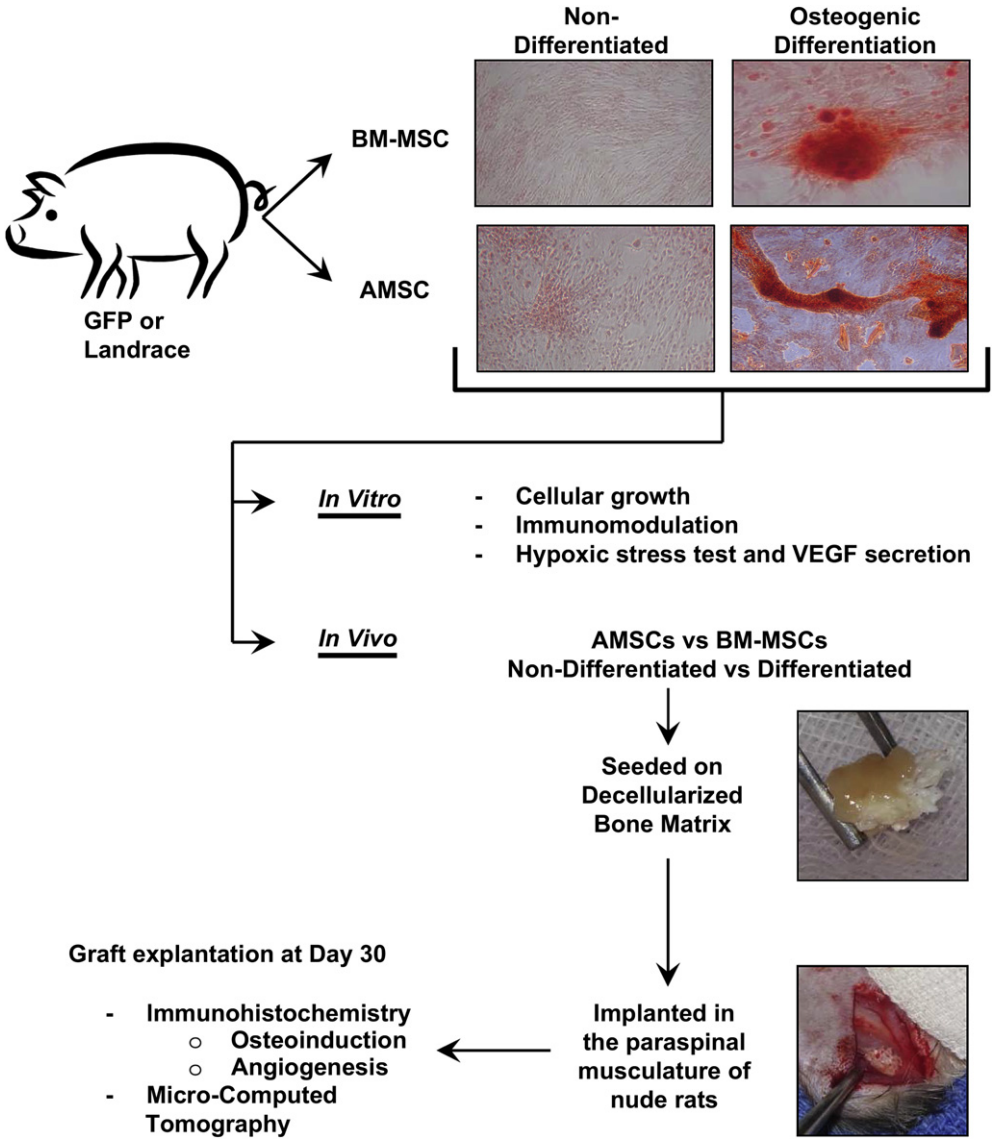

Fig. 1. Experimental protocol for pig AMSCs and BM-MSCs. In vitro assays investigated the capacity of cellular proliferation and differentiation, MLR for immunomodulation assessment, and hypoxic stress for VEGF secretion assays. In vivo experiments consisted of the implantation procedure of nondifferentiated and differentiated cells, issued from BM-MSCs or AMSCs cultures, seeded on a decellularized bone scaffold and implanted in the paravertebral musculature of nude rats. Implants were assessed by immunohistochemistry and micro-CT analysis to quantify angiogenesis and osteogenesis.