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Human adipose-derived stem cells isolated from young and elderly women: their differentiation potential and scaffold interaction during *in vitro* osteoblastic differentiation

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Background aims

Several authors have demonstrated that adipose tissue contains multipotent cells capable of differentiation into several lineages, including bone, cartilage and fat.

Methods

This study compared human adipose-derived stem cells (hASC) isolated from 26 female donors, under 35 and over 45 years old, showing differences in their cell numbers and proliferation, and evaluated their in vitro *adipocytic and osteoblastic differentiation potential.*

Results

The cellular yield of hASC from older donors was significantly greater than that from younger donors, whereas their clonogenic potential appeared slightly reduced. There were no significant discrepancies between hASC isolated from young and elderly women regarding their in vitro *adipocytic differentiation, whereas the osteoblastic potential was significantly reduced by aging. We also assessed the influence of hydroxyapatite (HAP) and silicon carbide (SiC–PECVD) on hASC. Even when cultured on scaffolds, hASC from younger donors had better differentiation into osteoblast-like cells than hASC from older donors; their differentiation ability was up-regulated by the presence of HAP, whereas SiC–PECVD produced no significant effect on hASC osteoblastic differentiation.*

Conclusions

The large numbers of hASC resident in adipose tissue and their differentiation features suggest that they could be used for a successful bone regeneration process in vivo. *We have shown that age does not seem to affect cell viability and* in vitro *adipocytic differentiation significantly, whereas it does affects osteoblastic differentiation, in the absence and presence of two-dimensional and three-dimensional scaffolds.*

Keywords

Aging, ex vivo *expansion, human adipose-derived stem cells, multipotential differentiation, osteoprogenitor cells, tissue regeneration.*

Introduction

Because of a low self-regenerative faculty, bone and cartilage defects caused by trauma, tumors and congenital deficiency never restore spontaneously. Innovative musculoskeletal regeneration approaches include tissue-engineering techniques using adult mesenchymal stromal cells (MSC) [1–10]. Bone marrow (BM), muscle, synovial membrane and adipose tissue are sources of MSC [11–14]. It is known that adipose tissue changes during life, together with a coordinated remodeling of the adipose vascularization [15];

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these modifications are mediated by resident stem cells [15]. Many authors have shown that these multipotent cells share quite similar features with BM stromal cells (BMSC) [13,16,17]; indeed, human adipose-derived stem cells (hASC) are clonogenic, proliferate easily and their progenies show a broad multilineage differentiation potential [18–21]. Furthermore, hASC proliferation and differentiation do not appear to be negatively affected by donor age, in contrast to reports on BMSC [22,23]. Compared with other adult tissues, adipose tissue has several advantages for both research purposes and clinical applications: a minimally invasive procedure is required for tissue withdrawal and a large number of multipotent cells can be isolated rapidly.

Musculoskeletal tissue regeneration often requires the combination of cells and scaffolds. The ideal material should be bio-degradable or bio-absorbable, suitable for cellular adhesion, and not induce inflammatory and immunologic responses [2]. In particular, in bone tissue engineering, the biomaterial should provide biomechanical support until tissue regeneration is completed and, during this process, the scaffold needs to disappear progressively to allow new tissue formation and cell colonization. Loading stem cells on scaffolds *in vitro* before implantation may lead to a faster bone formation and osteointegration *in vivo* [24,25].

We compared hASC isolated from female donors who were under 35 and over 45 years old, and examined their *in vitro* adipocytic and osteoblastic differentiation potential. We also investigated the influence of natural and synthetic scaffolds on hASC osteoblastic differentiation.

Methods

Isolation and culture expansion of hASC

The study was carried out after institutional review board approval. Subcutaneous fat was obtained from 26 healthy female donors [age range 21–68 years, body mass index (BMI) <30, without any pathologic obesity] undergoing plastic surgery by elective lipoaspiration, after written consent. Primary cultures of the stromal vascular fraction (SVF) were established as described previously [13]. Briefly, the raw lipoaspirates (50–100 mL) were washed at least three times with phosphate-buffered saline (PBS); the matrix was then enzymatically digested with 0.075% type I collagenase (Worthington, Lakewood, NJ, USA) at 37°C with continuous agitation for 30 min. The SVF was then centrifuged (1200 g, 10 min) and filtrated through a sterile medication lint. The collected SVF cells were plated in control medium [Dulbecco's modified Eagle medium (DMEM) +10% fetal bovine serum (FBS) supplemented with 50 U/mL penicillin, 50 μg/mL streptomycin and 2 mM L-glutamine] at approximately 10^5 cells/cm^2. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2. After 48 h non-adherent cells were removed and the medium was changed every other day. Adherent cells were grown until 80% confluence, detached with 0.5% trypsin/0.2% EDTA (Ethylene Diamine Tetraacetic acid), and replated at a density of 10^4 cells/cm^2 for further analyzes.

For the raw fat manipulation study, lipoaspirates were separated into two experimental groups, 'filtered' and 'centrifuged', according to the intra-operative manipulation procedure, as described previously [26–28]. The hASC isolation was performed as described above.

Fibroblast and osteoblast colony-forming unit assays

A colony-forming unit–fibroblast (CFU-F) assay was performed as described previously [29] with minor modifications. hASC were plated in six-well plates at low density by limiting dilution (starting dilution 48 cell/cm^2, ending dilution 1 cell/cm^2) and cultured at 37°C in a humidified atmosphere with 5% CO_2 in DMEM/20%FBS. After 6 days the medium was replaced, and 10 days after cells were fixed with methanol and stained with Gram's crystal violet. The frequency of CFU-F was established by scoring the individual colonies and expressed as a percentage relative to the seeded cells.

A colony-forming unit–osteoblast (CFU-O) assay was performed by plating cells in six-well plates by limiting dilution and culturing at 37°C in osteogenic medium (as described below) for 14 days. Colonies were then stained with Alizarin Red S (pH 4.1).

Cell lineage differentiation and evaluation of differentiation markers

Osteoblastic differentiation

10^4 hASC/cm^2 were induced to differentiate on monolayer in osteogenic medium consisting of control medium supplemented with 10 mM glicerol-2-phosphate, 10 nM dexamethasone, 150 μM L-ascorbic acid-2-phosphate and 10 nM cholecalciferol. After 14 days of differentiation, alkaline phosphatase activity (ALP) was determined (Triton X-100 0.1% in ddH_2O as lysis buffer) using 1 mM p-nitrophenylphosphate in alkaline buffer (100 mM diethanolamine and 0.5 mM $MgCl_2$, pH 10.5) as substrate [30] and normalized

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