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Qualitative and quantitative differences of adipose-derived stromal cells from superficial and deep subcutaneous lipoaspirates: a matter of fat

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Abstract

Background aims. Subcutaneous fat represents a valuable reservoir of adipose-derived stem cells (ASCs) in the stromal vascular fraction (SVF), widely exploited in regenerative medicine applications, being easily harvested through lipoaspiration. The lack of standardized procedures for autologous fat grafting guided research efforts aimed at identifying possible differences related to the harvesting site, which may affect cell isolation yield, cell growth properties and clinical outcomes. Subcutaneous fat features a complex architecture: the superficial fascia separates superficial adipose tissue (SAT) from deep layer tissue (DAT). We aimed to unravel the differences between SAT and DAT, considering morphological structure, SVF composition, and ASC properties. *Methods.* SAT and DAT were collected from female donors and comparatively analyzed to evaluate cellular yield and viability, morphology, immunophenotype and molecular profile. ASCs were isolated in primary culture and used for *in vitro* differentiation assays. SAT and DAT from cadaver donors were also analyzed through histology and immunohistochemistry to assess morphology and cell localization within the hypoderm. *Results.* Liposuctioned SAT contained a higher stromal tissue compound, along with a higher proportion of CD105-positive cells, compared with DAT from the same harvesting site. Also, cells isolated from SAT displayed increased multipotency and stemness features. All differences were mainly evidenced in specimens harvested from the abdominal region. According to our results, SAT features overall increased stem properties. *Conclusions.* Given that subcutaneous adipose tissue is currently exploited as the gold standard source for high-yield isolation of adult stem cells, these results may provide precious hints toward the definition of standardized protocols for microharvesting.

Key Words: *subcutaneous adipose tissue, adipose-derived stem cells, regenerative medicine, stemness*

Introduction

Adipose tissue (AT) is attracting the scientific community as a valuable tool for regenerative medicine approaches. Indeed, rather than being exclusively an energy reservoir, AT comprises many cell populations [1,2]. Its stromal vascular fraction (SVF) is part of the basic supporting framework, provides collagen fibers and blood vessels, and represents a reservoir of multipotent stem cells—namely, adipose-derived mesenchymal stem cells (ASCs) [3–7]. ASCs display an immunophenotype similar to that of bone marrow mesenchymal stem cells and express a molecular profile that is consistent with their stemness and uncommitted status [8].

The high yield of stem cells qualifies liposuctioned AT as a suitable source of autologous adult multipotent stem cells, also based on its ubiquity, easy retrieval, large quantity and minimally invasive harvesting procedure [9].

Of note, however, is that because of the discrepant biological properties found in different depots, fat is not all the same [10,11]. Higher number of ASCs reside in subcutaneous depots compared with visceral fat [12],

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and further differences can be described within the subcutaneous AT pad (ie, the hypoderm). This features a complex architecture, originally described long ago [13]; basically, the superficial fascial system (SFS) separates the subcutaneous AT into two layers: the areolar layer or superficial AT (SAT) and the lamellar layer or deep AT (DAT) [14–19]. SAT is localized right below the skin and is formed by small fat lobules tightly packed between fibrous septae, deriving from the SFS, oriented perpendicularly to the skin. DAT lies below the fascia superficialis and consists of large fat lobules, loosely packed within widely spaced vertical and oblique fibrous septae. The areolar AT is widely represented all over the body, whereas the lamellar tissue is more represented in specific body areas: abdomen, hips, peritrochanteric region and internal surface of the upper third of thigh, knees and posterior surface of the arm [20–26].

AT is commonly harvested from subcutaneous depots through lipoaspiration; the harvesting site is believed to affect cell isolation yield along with cell growth properties [27,28]. The amount of SVF isolated from subcutaneous liposuction aspirates and the proportion of stem-progenitor cells contained varies widely across individuals on the basis of several factors, including age, harvesting sites, among other factors [28–32].

Besides SVF and ASCs isolation, AT is used worldwide for fat grafting or autologous free fat transfer, in both cosmetic and reconstructive surgery, although the lack of standardized procedures causes inconsistent and variable clinical outcomes [9,33,34]. Each step in the transfer process (harvesting technique, aspiration cannula, preparation protocol and injection method) can cause mechanical damage to the grafted tissue, affecting tissue viability and postoperative outcomes. The clinical outcomes are also believed to be affected by the liposuction donor site because of the variable yield of stem/progenitor cells [35]. The common practice for selecting the liposuction site is generally based on the individual experience of the plastic surgeon or on patient need or choice [27].

Aimed at defining possible hints toward a standardization of fat grafting techniques, most scientific studies so far have addressed the comparisons between AT harvesting sites, focusing on different anatomical regions, regardless of the presence of the SFS. This study was intended to demonstrate the differences between the SAT and DAT subcutaneous layers with regard to morphological structure, SVF composition and ASCs properties, focusing on molecular pathways involved in stemness maintenance.

Methods

All the chemicals were purchased from Sigma-Aldrich unless otherwise specified.

Patients

Sixteen female patients undergoing first-time liposuction for elective breast fat grafting, were enrolled at our institution after signing a written informed consent. The entire protocol was approved by the Ethics Committee of the Università Cattolica del Sacro Cuore in Rome, Italy. All patients were in the 35- to 48-year age range (mean age 42 years, median age 43), had a normal weight (mean body mass index 22 kg/m^2; body mass index range: 18.5–24.9 kg/m^2) and were nonsmokers.

Abdomen, inner knee and external thigh were identified as sites for fat grafting. AT was harvested under general anaesthesia through ultrasound-assisted liposuction, according to standardized technique [36–39], using the superwet method [40,41]. SAT and DAT hypoderm specimens were collected separately [42].

Specifically, liposuction was performed operating a constant pressure of 500 mm Hg, using a Mercedes liposuction cannula (1.8 mm diameter with a bullet-like tip and 0.9-mm holes). Superficial or areolar subcutaneous fat (SAT) specimens were collected from the right-side abdomen, knee and thigh region; deep or lamellar subcutaneous fat (DAT) was collected from the left-side regions. Hence, six specimens of subcutaneous AT were harvested from each patient, resulting in overall 96 specimens: 48 SAT and 48 DAT. Care was taken to avoid cross-contamination of AT specimens. Five-cubic-centimeter aliquots of each lipoaspirate specimen were used in the study, transferred to sterile tubes, appropriately labeled and immediately sent to the laboratory setting.

In addition, three cadaver donors were made available from the Institute of Pathology of the same institution and used for the collection of full-thickness skin specimens from the abdominal wall, performed during standard dissection. These specimens were used for histological analysis and immunohistochemistry of the hypodermal layers.

Morphological analysis of SAT and DAT

For histology on liposuctioned AT from patients, an aliquot of each AT specimen was smeared on a slide, fixed with Cytofix fixation buffer (FIRMA) and stained with Sudan III (Carlo Erba Reagents). A distinct aliquot of each specimen was fixed in 4% formaldehyde isotonic phosphate-buffered saline for 2 h, embedded in paraffin and sectioned into 4-μm slices. The sections were stained with Hematoxylin (Dako) and Azan Trichrome (Bio-Optica). The staining results were analyzed with a Zeiss Axiophot microscope.

Abdominal skin tissue specimens collected from cadavers was used for standard histology and