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Age influence on stromal vascular fraction cell yield obtained from human lipoaspirates

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Abstract

Background aims. The adipose stromal vascular fraction (SVF) is a heterogeneous population of mononuclear cells that includes approximately 1–10% mesenchymal stromal cells. These SVF cells can be freshly obtained from human lipoaspirates and represent and ideal candidate for regenerative medicine applications. In the present study, we analyzed the SVF yield as a function of the patient's age. *Methods.* Adipose tissue samples from 52 informed subjects (all women) were processed by means of an innovative point-of-care technology for SVF isolation (GID platform). After enzymatic dissociation of adipose tissue and SVF pellet resuspension, we measured the concentration of mononucleated cells as well as other cell quality analyses on the cell suspension obtained. We then calculated the cell yield as total nucleated cells per milliliter of dry adipose processed. *Results.* The mean SVF yield obtained was $7.19 \times 10^5 \pm 2.11 \times 10^5$ total nucleated cells per milliliter of adipose tissue. Our results show that there is a clear statistically significant decline in SVF cell yield with increasing age. *Conclusions.* Because all samples were obtained from the same donor area and the isolation technique used was the same in all cases, we conclude that the SVF cell yield in women is affected by patient age. Specific age-related factors should be analyzed in the future to explain these results.

Key Words: *adipose, aging, cell yield, lipoaspirate, stromal vascular fraction*

Introduction

There is increasing evidence that addition of supporting stromal cells to its parenchyma results in potentiation and/or repair of tissue functions (1). There are many ways to obtain stromal cells from tissues, and, among them, the enzymatic dissociation is the most efficient and reliable procedure. Adipose tissue is the best candidate from which to obtain stromal cells because it is an abundant tissue, it is easily accessible and is a rich source of SVF cells useful for regenerative medicine (2).

For cell-based therapies, an advantageous approach would be to harvest the autologous SVF cells and return them back to the patient within a single surgical procedure, thereby avoiding lengthy and costly *in vitro* cell culture expansion. Many clinical ongoing studies use this cell population to treat patients (3).

Multiple platforms have emerged to enable the adipose tissue dissociation process; each one performs differently (4,5). In the present study, we used the GID platform (The GID Group Inc, Louisville, CO, USA), by which we standardize physical and physiological conditions of adipose tissue before SVF isolation with the use of a consistent and repeatable method.

The aging process is known to affect regeneration capability of adult tissues. Adipose tissue is also affected by age-related changes, and adipose tissue–derived mesenchymal cell number, differentiation potential and angiogenic functional capacity are affected by age (6,7). However, there are opposing or controversial results related to mesenchymal cell yield after adipose tissue processing in aged human patients (8–10).

As we gathered information during technology validation, it became apparent that there were substantial differences in cell yield performance, irrespective of donor site, processing method or tissue sampling error. On the basis of these observations, we established the hypothesis that subject's age would influence the stromal SVF cell yield on adipose tissue samples. The stromal tissue compartment, which is dissociated by enzymatic digestion, allows us to achieve a reproducible number of SVF cells contained in liposuctioned adipose tissue regardless of the anatomic

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site for tissue harvest. Because little is known about the impact of age on cell yield after adipose SVF isolation, we present our results regarding the impact of age in SVF cell yield in female patients.

Methods

Patients

Human adipose tissue samples for SVF isolation were obtained from healthy women (subcutaneous abdominal depots) undergoing cosmetic and reconstructive surgeries involving cell-enriched fat grafts. This study was performed in 52 female patients from 2010–2012: in the breasts for 48 patients (cosmetic breast augmentation, breast reconstruction after mastectomy, tuberous breast deformities or breast asymmetry), the buttocks for two patients (augmentation or reconstruction) and the face for two patients (filling or facial rejuvenation). The protocol was approved by the local ethics committee. Informed consent was signed by all patients. All procedures took place at Palmaplanas Clinic (Palma de Mallorca, Spain) and were performed by the same plastic surgeon.

All patients were white women with a mean body mass index (BMI) of 18.59. Their ages ranged from 19–71 years (mean, 40.12). The demographic and surgical data for these patients are summarized in Table I and Table II.

Adipose tissue harvesting and processing

Liposuction was performed under general anesthesia and sterile conditions. In all cases, fat tissue was harvested from the abdominal area with the use of a power-assisted lipo-aspiration system. The donor area was injected with tumescent solution: lidocaine (1%) and epinephrine (1:1000) in lactated Ringer (LR) solution and allowed 10–15 min to achieve hemostasis and anesthesia before proceeding with fat harvesting. The mean volume of tumescent solution injected was 1.5 L. Blunt cannulas 4 mm in diameter and 20 cm in length were used. The fat was harvested into either a GID 700 device for fat graft processing or GID SVF-1 for SVF isolation (Figure 1). Approximately half of the harvested volume was used for fat graft preparation and the other half for SVF isolation. All tissue-processing procedures were performed in sterile conditions inside the operating room by a skilled biologist.

Table I. Patient data.

	No. of cases: 52
Sex	All women
Age (years)	40.12 ± 13.53
BMI (Kg/m^2)	18.59 ± 2.19
Raw lipo-aspirate volume, range	1.5–3 L
Fat graft injection volume, range	50–375 mL
Adipose tissue volume for SVF isolation	100–300 mL

Values are mean ± standard deviation.

Table II. Age distribution of patients analyzed in the study.

Age group	No. of patients
<30 years	11
30–40 years	13
40–50 years	11
50–60 years	9
>60 years	8

Protocol for SVF isolation with use of the GID SVF-1 device

The sterile GID tissue canister containing the harvested adipose tissue was moved to a sterile location in the operating room for processing. Briefly, the adipose tissue was washed three times with the use of LR and the mechanical/filtration system inside the device; any remaining fluids were aspirated, and the amount of dry adipose tissue was weighed. The same volume of digestion buffer (1:1 ratio with dry adipose mass) containing dissociation enzyme (GIDzyme-2, 200 cdu-collagen digestive units-/mL) was added to the canister, and the canister was incubated at 37°C for 40 min with constant shaking at 150 rpm. After digestion, the enzyme activity was stopped with the use of human albumin (2.5% final concentration), and the device was centrifuged at 800*g* for 10 min. After supernatant removal, the resulting cell pellet was resuspended with the use of LR and a 14-gauge spinal needle and was then loaded into a 20-mL syringe. An aliquot was taken for cell quality and safety analyses.

All cell suspensions obtained were analyzed by means of image cytometry with the use of the nucleocounter NC-3000 (Chemometec, Denmark) for cell count and viability, cell cycle and mitochondrial potential assay, following the manufacturer's instructions. To determine viability and cell concentration, a diluted sample of the cell suspension is loaded into a Via1-Cassette, which is coated with two different dyes (acridine orange and 4′-6-diamidino-2-phenylindole), staining the entire nucleated cell population and the non-viable nucleated cells, respectively. The apoptotic index was analyzed by means of the NC-3000 mitochondrial potential assay with the use of JC-1 dye, following the manufacturer's instructions (Chemometec).

Additionally, the cell suspension was assayed for endotoxin levels with the use of the Endosafe-PTS device (Charles River Laboratories International, Inc, France). A cell suspension sample diluted in endotoxin-free water was loaded into the cartridge.