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Original article

Isolation of autologous adipose tissue-derived mesenchymal stem cells for bone repair

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ABSTRACT

Introduction: Adipose tissue represents an abundant and accessible source of adult stem cells that can differentiate into cells and tissues of mesodermal origin, including osteogenic cells.
Methods: This paper describes the procedure to obtain a 5-cm^3 saline sample, containing the adipose-derived stem cells (ASCs) pellet, starting from lipoaspirate obtained from a conventional abdominal liposuction.
Results: A mean of 2.5×10^6 cells is isolated for each procedure; 35% (875000) of these are CD34+/CD45− cells, which express a subset of both positive (CD10, CD13, CD44, CD59, CD73, CD90, HLAABC) and negative (CD33, CD39, CD102, CD106, CD146, HLADR) cell-associated surface antigens, characterizing them as ASCs.
Conclusions: This procedure is easy, effective, economic and safe. It allows the harvesting of a significant number of ASCs that are ready for one-step bony regenerative surgical procedures.

1. Introduction

Mesenchymal stem cells (MSCs) have recently been investigated as a possible powerful tool for aiding bone repair in peculiar indications [1–5]. Bone marrow-derived MSCs (BM-MSCs) have been shown to be useful in both pre-clinical [3,6,7] and clinical [1,7,8] studies, with and without cell culturing and with or without a matrix. Major indications include large segments of bone lost owing to trauma (requiring reconstruction), tumors and skeletal deformities (requiring resection), avascular necrosis, and non-unions or delayed unions [1,2,4,9,10]. Specifically, delayed unions and non-unions are still frequent and major complications of the treatment of fractures, and cell therapy may represent a valid and better alternative to the use of bone grafting procedures or, simply, osteoconductive substitutes [2,9]. Several researchers have described the similarity between BM-MSCs and adipose-derived stem cells (ASCs), as well as the potential use of ASCs in bone regeneration [4,5,11,12].

Subcutaneous adipose tissue is an attractive reservoir of progenitor cells because it is easily accessible, abundant and self-replenishing. The stromal vascular fraction (SVF) of adipose tissue consists of a heterogeneous mixture of cells, including endothelial cells, smooth muscle cells, pericytes, leukocytes, mast cells, and pre-adipocytes [11,13–15]. In addition to these cells, the SVF contains an abundant population of multipotent ASCs that have the capacity to differentiate into cells of mesodermal origin "in vitro", e.g. adipocytes, chondrocytes, osteoblasts, and cardiomyocytes [11,13–19].

In the present paper, we describe a practical protocol for harvesting and isolating autologous ASCs, ready to be used for intraoperative cell therapy for bone repair.

2. Materials and methods

In the immediate preoperative period, a single dose of 1.5 g of ampicillin sodium + sulbactam sodium was given intravenously. The procedures were performed under sedo-analgesia using the tumescent technique (Fig. 1A), infiltrating the subcutaneous tissue of the abdominal region with Klein solution [20]: 0.9% NaCl 1000 mL, lidocaine 1000 mg (0.1%), 8.4% sodium bicarbonate 15 mL, and adrenalin 0.5 mg (concentration 1:2000000). The volume of injected fluid was proportional to the estimated volume to be extracted by liposuction (usually 100 cm^3). Next, a conventional

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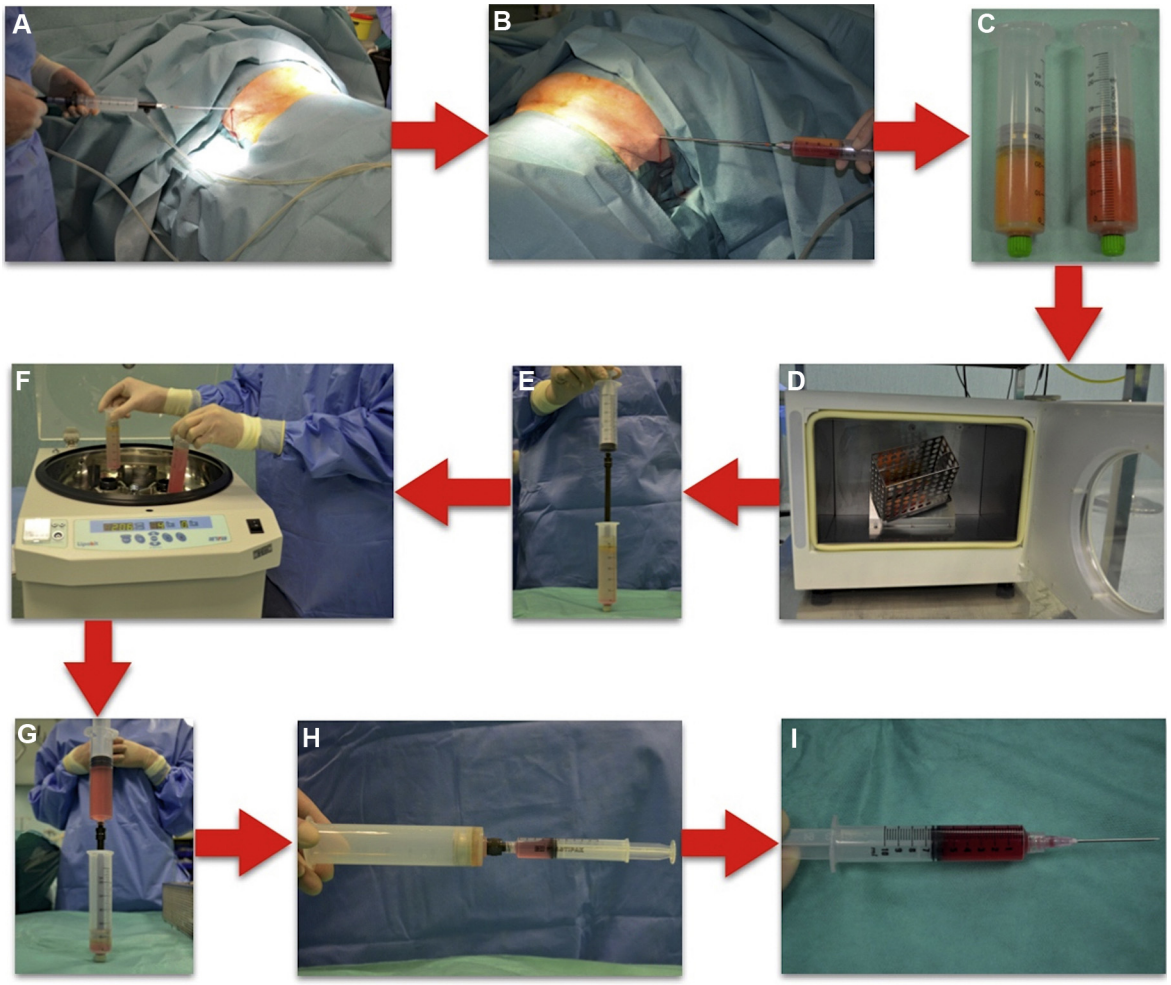

Fig. 1. Steps for ASCs standardised isolation technique. Step 1: after conventional infiltration (A) and liposuction (B), the harvested fat is ready (C) to be mixed with a collagenase digestion solution; further steps are incubation (D) for 30 min. At 37 °C, washing (E) with saline solution to remove collagenase, centrifuge (1600 RPM × 6 min.) (F) and further washing (G, H) to obtain the ASCs pellet (I).

abdominal liposuction was performed using 2-mm blunt cannulas (Fig. 1B). The devices used (Lipokit-Celltibator, Medi-Khan Inc., USA) were designed for tumescent injection, aspiration, harvesting, filtering, and processing of autologous fat tissue in a closed circuit, thus avoiding any risks of contamination. However, the same procedure might just as easily be performed with cannulas connected to Luer-Lock 50 cm^3 syringes. The lipoaspirate was then processed according to the following procedure: about 50 mL of highly concentrated adipose tissue (Fig. 1C) was mixed rapidly with 1 g of collagenase (Collagenase NB 6 GMP Grade #17458, Serva GmbH, Heidelberg, Germany), previously diluted with 50 mL of sterile phosphate-buffered saline (PBS). The resultant cellular suspension (lipoaspirate + collagenase solution) was then incubated for 30 min (Fig. 1D) at 37 °C. Subsequently, only 10 mL of solution (containing the SVF pellet) remained; this solution was then washed three times (Fig. 1E) in 45 mL saline solution. After each wash, the syringes containing the SVF were positioned inside the centrifuge at 200 RCF × 4 min (Fig. 1F). At the end of the procedure, a 5-cm^3 saline sample, containing the ASC pellet, was ready for its application in regenerative medicine (Fig. 1G–I). The whole procedure lasted about 120 min.

Processed lipoaspirates from three healthy patients were studied. Surface markers of the isolated ASCs were analyzed by flow cytometry according to the criteria for defining multipotent mesenchymal stromal cells, as defined by the International Society for Cellular Therapy [21]. Following our previously described protocol [22], the cells were incubated for 20 min and stained with antibodies specific for human ASC markers: CD10, CD13, CD31, CD33, CD34, CD39, CD44, CD45, CD59, CD73, CD90, CD102, CD106, CD146, HLADR, and HLAABC (Becton, Dickinson and Co., NJ, USA). The cells were analyzed using an eight-color flow cytometer (FACSCanto II; Becton, Dickinson and Co., NJ, USA). The dye 7-amino-actinomycin D (7-AAD) was added to assess the viability of the cells. At least 20,000 events were collected.

3. Results

A mean of 2.5×10^6 cells was isolated per procedure: 35% (875,000) of these were CD34+/CD45− cells (Fig. 2A), which expressed a subset of both positive (CD10, CD13, CD44, CD59, CD73, CD90, HLAABC) and negative (CD33, CD39, CD102, CD106, CD146, HLADR) cell-associated surface antigens, characterizing them as ASCs; 60% (Fig. 2B) were CD34+CD45−CD31− cells (20% total cells; 525,000 cells), and 40% were CD34+CD45−CD31+ cells (14% total cells; 350,000 cells). The overall viability of these cells was equal to 93% (Fig. 2C).

4. Discussion

The harvesting of BM-MSCs has practical constraints. These include pain at the harvest site, and harvest of only a small volume of bone marrow (and therefore a small number of MSCs),