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A Standardized Method of Isolating Adipose-Derived Stem Cells for Clinical Applications

Edoardo Raposio, MD, PhD, Giorgia Caruana, MD,* Maira Petrella, MSc,* Sabrina Bonomini, PhD,† and Michele P. Grieco, MD**

Abstract: White adipose tissue is the most abundant and accessible source of stem cells in the adult human body. In this paper, we present a standardised and safe method of isolating and maximizing the number of adipose-derived stem cells (ASCs) from conventional liposuction for clinical applications, which was carried out through both mechanical (centrifuge) and enzymatic (collagenase) means. Isolated cells were characterized through flow cytometry assay. Gathered data showed a greater amount (9.06×10^5 ASCs from 100 mL of adipose tissue) of isolated ASCs compared to previous protocol, also with high (99%) cell vitality; the procedure we presented is easy and fast (80 minutes), allowing collecting a significative number of mesenchymal stem cells, which can be used for clinical purposes, such as wound healing.

Key Words: adipose-derived stem cells, regenerative medicine, collagenase, wound healing

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To date, albeit the best characterized adult stem cell population considered to possess multipotent capacity is that of bone marrow mesenchymal stem cells (MSCs),[1] white adipose tissue has been given attention for being the most abundant and accessible source of stem cells in the adult human body.[2] By definition, adipose-derived stem cells (ASCs) are plastic-adherent, proliferative, multipotent cells, isolated from adipose tissue and able to exist in an undifferentiated state, also undergoing self-renewal and multilineage differentiation, leading to terminally differentiated cells.[2–4] For these potentials, and because they can be easily harvested in a great amount with minimal donor site morbidity, ASCs proved to be particularly promising for regenerative therapies.

It is noteworthy though that there is not a standardized protocol to isolate ASCs for clinical application, which led to an inconstancy in literature.[1,4,5] To date, there is also a shortage in clinical reports involving ASCs in cell therapy on humans[6]: works available in literature mostly used basic research–derived protocols and/or other applications than clinical ones. Hence, there is a need of a standardized method for clinical purposes, which optimize and unify process schedule and isolation procedure, as well as the whole tissue manipulation.

With this study, we suggest a standardized method of isolating and maximizing the number of ASCs from conventional liposuction[7] for clinical applications, which was carried out through both mechanical (centrifuge) and enzymatic (collagenase) means.

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From the*Department of Surgical Sciences, Plastic Surgery Division, University of Parma; Cutaneous, Mininvasive, Regenerative and Plastic Surgery Unit; and †Division of Hematology, Department of Clinical and Experimental Medicine, Parma University Hospital, Parma, Italy.

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AQ1 Reprints: Edoardo Raposio, MD, PhD, Plastic Surgery Division, Department of Surgical Sciences, University of Parma, Via Gramsci 14, 43126, Parma, Italy. E-mail: edoardo.raposio@unipr.it.

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MATERIALS AND METHODS

The operating room was set up with a centrifuge (Lipokit; Medikhan, Korea) and an incubator (Celltibator; Medikhan, Korea). One hundred milliliters of lipoaspirate were harvested from healthy patient and processed to obtain the stromal vascular fraction (SVF) in which ASCs could be found. A first centrifuge at 1600 rounds per minute × 6 minutes allowed us to obtain about 50 ml of high quality concentrated adipose tissue, which was suddenly mixed with 50 mL collagenase digestion solution (Collagenase NB 6 GMP Grade 17458; Serva GmbH, Heidelberg, Germany), previously diluted with sterile phosphate-buffered saline (PBS) as follows: 1 g of collagenase was suspended in 10 mL PBS, and 1 mL of the obtained solution was further added with 49 mL of PBS. The solution obtained (lipoaspirate + collagenase digestion solution) was then incubated for 30 minutes at 37°C in a shaker-incubator (Celltibator; Medikhan) and, after that, it was centrifuged at 200 relative centrifuge force × 4 minutes. Subsequently, only 10 mL of SVF were left and washed 2 times, each one with 45 ml saline solution. After each washing, syringes containing SVF were positioned inside the centrifuge at 200 relative centrifuge force × 4 minutes. The cellular pellet at the bottom of the syringe was then ready for use, vehiculated by 5 mL of saline solution. The AQ2 whole isolation procedure has taken about 80 minutes (Fig. 1), always F1 preventing SVF from air contact thus any eventual risk of contamination: indeed, a completely close circuit made of connectors allowed SVF and ASCs proceeding from syringe to syringe until ASCs were injected into the patient's area of interest (e.g. foot ulcer).

Characterization Of Ascs

Isolated cells were characterized by flow cytometry assay, according to our previously published protocol.[8]

RESULTS

The ASC markers[9] CD45−/CD34+/CD31− were detected in our ASC population (Fig. 2). The MSC marker CD90+ and endoglin marker F2 CD105+ were also found; because both CD90 and CD105 are endothelial population markers too, as suggested by Bourin et al,[9] we also looked for and found the presence of stromal cells marker CD73+ characterizing ASCs (Fig. 3). Starting from 100 mL of harvested fat tissue, we regularly F3 collected a mean of 9.06×10^5 ASCs (range, 8.4 to 9.72×10^5; SD ± 6.6×10^5), corresponding to 25.9% of the total number (mean of 3.5×10^6 cells; range, 3 to 4×10^6; SD ± 5×10^5) of isolated cells.

DISCUSSION

Current inconstancy in literature[1,4,5] about protocols for isolation of ASCs from fat tissue gave the scientific community the chance to thoroughly assess the pros and contras of different procedures, thus, perhaps, approaching more and more to a leading method. With their noteworthy work, Zuk et al[4] laid the groundwork for isolation of ASCs: during this procedure, washing of the adipose tissue, enzymatic digestion, centrifugation, and red blood cell lysis were performed sequentially. This method resulted in a fraction of stromal cells, including adipocytes, preadipocytes, endothelial cells, macrophages, fibroblasts, and a subpopulation of MSCs; although efficient, this procedure could be expensive and time-consuming when considered for clinical purposes.[10]

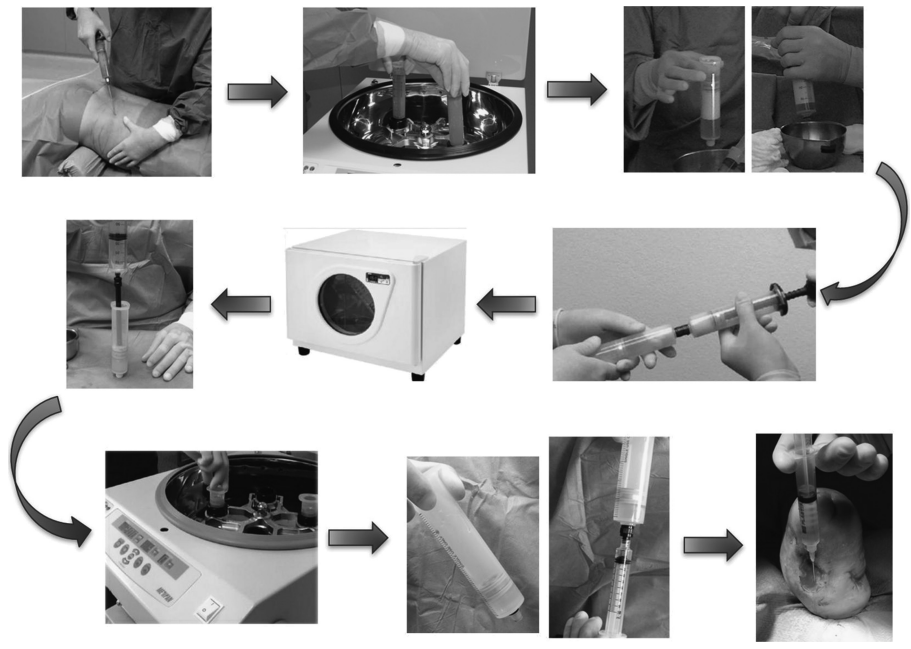

FIGURE 1. ASCs standardized isolation technique. Step 1: After conventional liposuction, the harvested fat tissue underwent a first centrifuge (1600 RPM × 6 minutes), obtaining concentrated adipose tissue, to be mixed with collagenase digestion solution (Collagenase NB 6 GMP Grade, 1 g/10 ml PBS). Step II: incubation for 30 minutes at 37°C, followed by centrifugation (400 RCF × 4 minutes). Step III: Washing with saline solution + centrifugation (400 RCF × 4 minutes), 2 times each. The ASCs pellet is ready for clinical use. RPM indicates rounds per minute; RCF, relative centrifuge force.

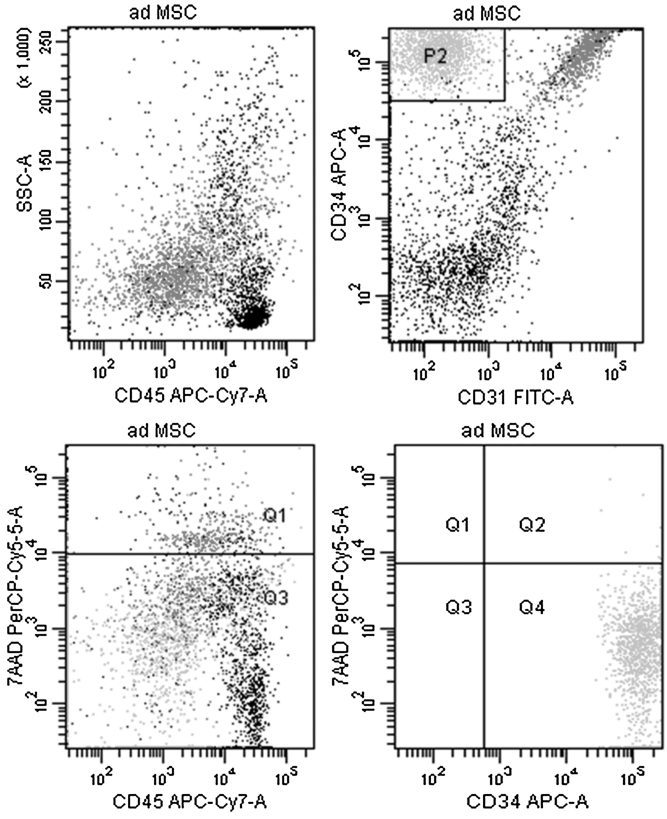

FIGURE 2. ASCs flow cytometric analysis: on the CD45– subset, CD34+ and CD31– distinguished stromal cells from the endothelial cells CD34+ and CD31+.

With our method, neither animal-derived serum, medium of any type, red blood cell–buffered solutions or trypsin are necessary; moreover, the procedure is carried out step by step in a closed circuit, always preventing ASCs from air contact, thus minimizing any eventual risk of contamination.

Gathered data showed the stem cells population we collected were 102.9% more abundant then previous standard enzymatic procedure[5], also with high cell vitality (99%).

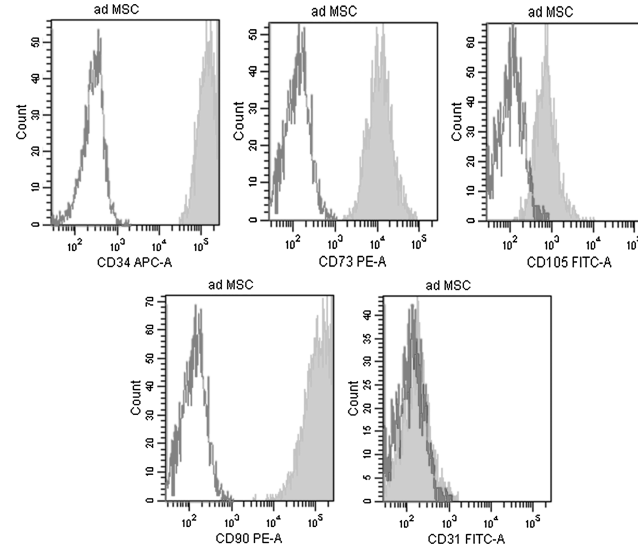

FIGURE 3. Flow analysis of human ASCs. Flow cytometric analysis showed that human ASCs cells did not express CD31, and CD45, but did express CD34, CD73, CD90, CD105. Red line, negative control. Green line, Ab expression. AQ3

The procedure we presented is easy and fast, allowing collecting a great amount (9.06×10^5 ASCs) of mesenchymal stem cells from adipose tissue, which can be used for clinical purposes, such as wound healing.

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AQ2 = "cc" was changed to "mL" Please check.

AQ3 = Please spell out Ab and also kindly verify if Figure 3 should appear in color, otherwise, modify the caption indicating color (green).

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