



University of Parma Research Repository

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

This is the peer reviewed version of the following article:

*Original*

A Standardized Method of Isolating Adipose-Derived Stem Cells for Clinical Applications / Raposio, Edoardo; Caruana, G; Petrella, M; Bonomini, S; Grieco, Michele Pio. - In: ANNALS OF PLASTIC SURGERY. - ISSN 0148-7043. - 76:1(2016), pp. 124-126. [10.1097/SAP.0000000000000609]

*Availability:*

This version is available at: 11381/2802882 since: 2016-12-06T15:05:39Z

*Publisher:*

Lippincott Williams and Wilkins

*Published*

DOI:10.1097/SAP.0000000000000609

*Terms of use:*

Anyone can freely access the full text of works made available as "Open Access". Works made available

*Publisher copyright*

note finali coverpage

(Article begins on next page)

**ARTICLE COVERSHEET**  
**LWW\_CONDENSED(7.75X10.75)**  
**SERVER-BASED**

Article : SPA50086

Creator : dsabac

Date : Wednesday July 29th 2015

Time : 01:50:14

Number of Pages (including this page) : 5

# 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 ( $9.06 \times 10^5$  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

(*Ann Plast Surg* 2015;00: 00–00)

To date, albeit the best characterized adult stem cell population considered to possess multipotent capacity is that of bone marrow mesenchymal stem cells (MSCs),<sup>1</sup> white adipose tissue has been given attention for being the most abundant and accessible source of stem cells in the adult human body.<sup>2</sup> 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.<sup>2–4</sup> 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.<sup>1,4,5</sup> To date, there is also a shortage in clinical reports involving ASCs in cell therapy on humans<sup>6</sup>: 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<sup>7</sup> for clinical applications, which was carried out through both mechanical (centrifuge) and enzymatic (collagenase) means.

## MATERIALS AND METHODS

The operating room was set up with a centrifuge (Lipokit; Medikhan, Korea) and an incubator (Cellticator; 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  $\times$  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 (Cellticator; Medikhan) and, after that, it was centrifuged at 200 relative centrifuge force  $\times$  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  $\times$  4 minutes. The cellular pellet at the bottom of the syringe was then ready for use, vehiculated by 5 mL of saline solution. The whole isolation procedure has taken about 80 minutes (Fig. 1), always 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 Asc

Isolated cells were characterized by flow cytometry assay, according to our previously published protocol.<sup>8</sup>

## RESULTS

The ASC markers<sup>9</sup> CD45–/CD34+/CD31– were detected in our ASC population (Fig. 2). The MSC marker CD90+ and endoglin marker CD105+ were also found; because both CD90 and CD105 are endothelial population markers too, as suggested by Bourin et al,<sup>9</sup> 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 collected a mean of  $9.06 \times 10^5$  ASCs (range,  $8.4$  to  $9.72 \times 10^5$ ; SD  $\pm 6.6 \times 10^5$ ), corresponding to 25.9% of the total number (mean of  $3.5 \times 10^6$  cells; range, 3 to  $4 \times 10^6$ ; SD  $\pm 5 \times 10^5$ ) of isolated cells.

## DISCUSSION

Current inconstancy in literature<sup>1,4,5</sup> 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<sup>4</sup> 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.<sup>10</sup>

Received April 20, 2015, and accepted for publication, after revision July 9, 2015.  
From the\*Department of Surgical Sciences, Plastic Surgery Division, University of Parma; Cutaneous, Minimally Invasive, Regenerative and Plastic Surgery Unit; and †Division of Hematology, Department of Clinical and Experimental Medicine, Parma University Hospital, Parma, Italy.

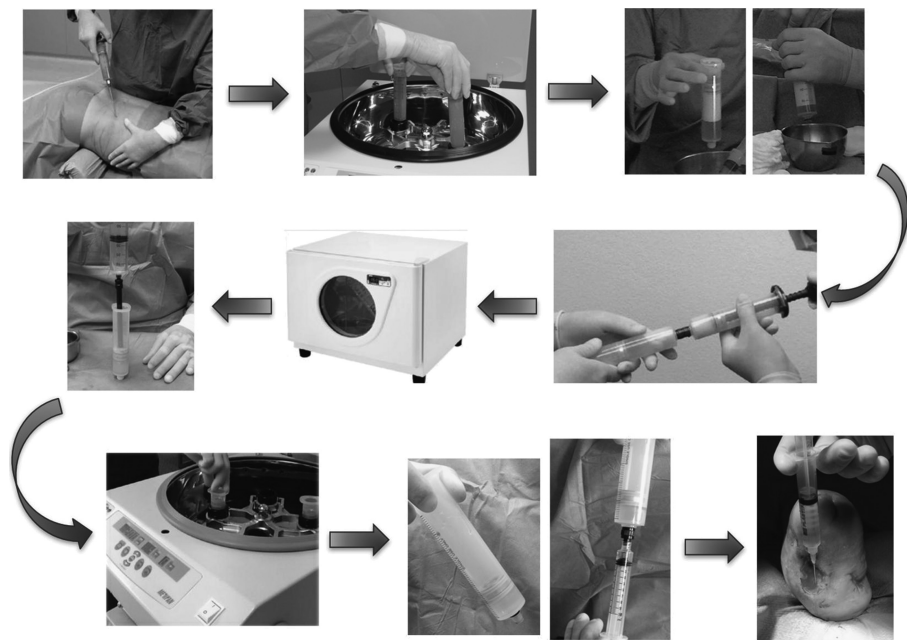
Conflicts of interest and sources of funding: Funding received for this work from National Institutes of Health (NIH), Wellcome Trust, Howard Hughes Medical Institute (HHMI), or others: none.

**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.

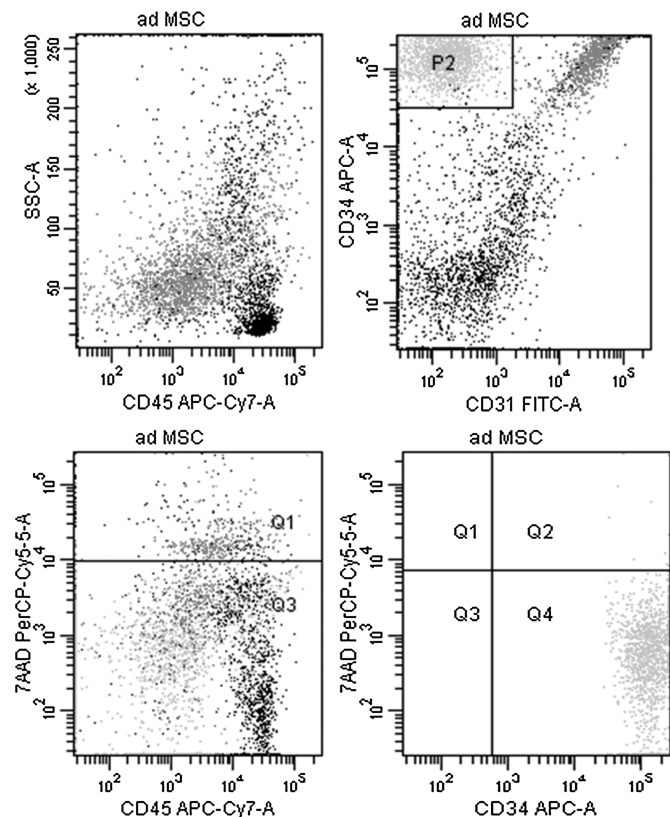
Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

ISSN: 0148-7043/15/0000–0000

DOI: 10.1097/SAP.0000000000000609



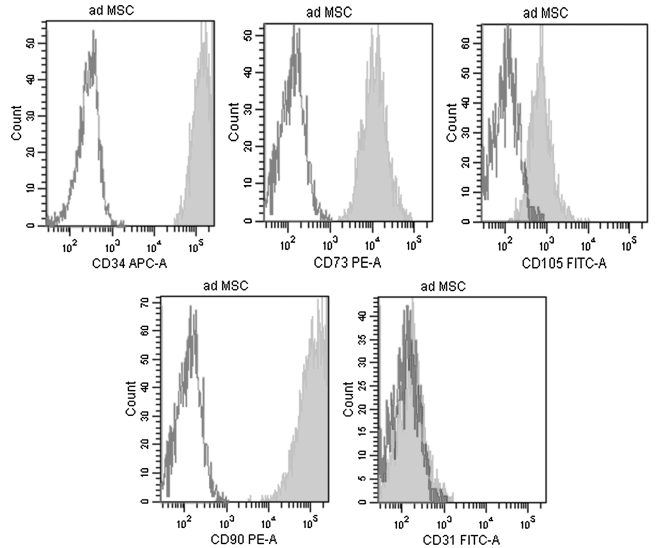
**FIGURE 1.** ASCs standardized isolation technique. Step I: After conventional liposuction, the harvested fat tissue underwent a first centrifuge (1600 RPM × 6 minutes), obtaining concentrated adipose tissue, to be mixed with collagenase digestion solution (Collagenase NB 6 GMP Grade, 1 g/10 ml PBS). 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 than previous standard enzymatic procedure<sup>5</sup>, 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.

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


## REFERENCES

1. Cheng KH, Kuo TL, Kuo KK, et al. Human adipose-derived stem cells: isolation, characterization and current application in regeneration medicine. *GMBHS*. 2011; 3:53–62.
2. Gimble GM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. *Circ Res*. 2007;100:1249–1260.
3. Bunnell AB, Flaat M, Gagliardi C, et al. Adipose-derived stem cells: Isolation, expansion and differentiation. *Methods*. 2008;45:115–120.
4. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng*. 2001;7:211–228.
5. Kitagawa Y, Koroby M, Toriyama K, et al. History of discovery of human adipose-derived stem cells and their clinical application. *Jpn J Plast Reconstr Surg*. 2006;49:1097–1104.
6. Trounson A, Thakar RG, Lomax G, et al. Clinical trial for stem cell therapies. *BMC Med*. 2011;9:52.
7. Flynn TC, Coleman WP, Field LM, et al. History of Liposuction. *Derm Surg*. 2000;26:515–520.
8. Raposio E, Caruana G, Bonomini S, et al. A novel and effective strategy for the isolation of adipose-derived stem cells: minimally manipulated adipose-derived stem cells for more rapid and safe stem cell therapy. *Plast Reconstr Surg*. 2014; 133:1406–1409.
9. Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy*. 2013;15:641–648.
10. Markarian CF, Frey GZ, Silveira MD, et al. Isolation of adipose-derived stem cells: a comparison among different methods. *Biotechnol Lett*. 2014;36:693–702.

## **AUTHOR QUERIES**

### **AUTHOR PLEASE ANSWER ALL QUERIES**

AQ1 = Please check if correspondence address is correct. 

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). 

**END OF AUTHOR QUERIES**