

# Adipose tissue derived stem cells: isolation, *in vitro* culture and potential uses in dermatology

## *Células-tronco derivadas de tecido adiposo: isolamento, cultivo in vitro e perspectivas de utilização em dermatologia*

### ABSTRACT

There are extensive reports on the characteristics and therapeutic potential of adult mesenchymal stem cells. The possibility of isolating them from excess adipose tissue obtained from other procedures is promising and extremely desirable. However, the protocols for such applications are still described in a summarized, rather than standardized, manner. Thus, the objective of this paper is to present a simple, practical and effective protocol for the collection of adipose tissue in the hospital environment, and for the isolation and *in vitro* cultivation of adult mesenchymal stem cells obtained from adipose tissue in a laboratory, with the purpose of discussing the potential uses of this technique in the field of dermatology.

**Keywords:** adult stem cells; dermatology; stem cells; adipose tissue ; tissue therapy.

### RESUMO

É extensa a literatura existente sobre as características e potenciais terapêuticos das células-tronco mesenquimais adultas, e a possibilidade de seu isolamento a partir de tecido adiposo excedente de outros procedimentos é promissora e extremamente desejável. Os protocolos para tal utilização, porém, ainda são descritos de maneira sumarizada e não padronizada. O objetivo dessa comunicação é apresentar um protocolo simples, prático e eficaz tanto da coleta do tecido adiposo em ambiente hospitalar quanto do isolamento e cultivo, em laboratório, *in vitro* de células-tronco mesenquimais adultas derivadas de tecido adiposo, com a finalidade de discutir as perspectivas da utilização dessa técnica na dermatologia.

**Palavras-chave:** células-tronco adultas; dermatologia; células-tronco; tecido adiposo; terapia celular.

### INTRODUCTION

The scientific innovation and therapeutic potential of stem cells derived from adult tissue for the scientific and medical fields has been discussed for several years. These adult stem cells (ASCs) appear to play a role in the tissues' natural renewal<sup>1,2</sup>. This therapeutic potential is based on intrinsic properties such as clonogenicity (the ability to detect low density cells and activate their duplication capacity), multipotentiality (the potential to originate a great number of more specialized cells), and autorenewal (the capacity to generate new multipotent cells).<sup>3-7</sup> Since these properties were highlighted and summarized in a *Science* magazine editorial in 1999<sup>8</sup>, the scientific community's interest in studying these cells has steadily grown, due to the enormous potential of application in tissue engineering and genetic therapy<sup>9</sup>.

## Review Article

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Studies related to cellular therapy are being increasingly published, revealing potential therapies for regenerative medicine – which focuses on the treatment of lesions and disorders – aimed at restoring the functions of damaged or lost cells and tissues through substitution or protection<sup>10</sup>. Currently, cellular therapy with adult mesenchymal cells is understood to offer advantages over other methods in tissue repair, resulting in high quality regeneration without the formation of scars or fibrosis. In addition, since it is an autologous transplant it presents a lower risk of rejection and transmission of disorders than other possible exogenous sources of stem cells, such as embryonal cells.<sup>3,4,10-15</sup>

Studies carried out by Meirelles and Nardi<sup>16</sup>, Haynesworth and others<sup>17</sup>, Majumdar and others<sup>18</sup>, Romanov and colleagues,<sup>19</sup> and Campagnoli et al.<sup>20</sup> demonstrated that mesenchymal stem cells (MSCs) isolated from bone marrow are capable of self-renovation and differentiation into a variety of conjunctive, osseous, adipose and muscular tissues lineages, allowing their operational classification. Meirelles and colleagues<sup>5</sup> showed that cells with MSC characteristics can be derived and cultured in vitro from different organs and tissues such as the brain, spleen, liver, kidneys, lungs, bone marrow, muscles, thymus and pancreas. The presence of stem cells in human adipose tissue had already been reported by Zuk and others<sup>21</sup>, who compared the differentiation potential of these cells with MSCs isolated from bone marrow and proved they are similar<sup>22</sup>.

It has already been demonstrated that a stem cell population derived from adipose tissue digested with collagenase – also called stromal vascular fraction – is capable of differentiating into several cellular lineages, including adipose tissue, cartilage, bone, skeletal musculature, neuronal cells, endothelial cells, cardiomyocytes and smooth muscle tissue cells<sup>3, 19, 20, 23-27</sup>. Because it is easy to obtain stem cells from adipose tissue with minimal patient discomfort – and many times with greater proliferative capacity than those derived from bone marrow cells – adipose tissue can be considered an ideal source of autologous stem cells. The greater proliferative capacity can be intrinsic to the cells or result from a greater density of stem cells in the initial population. Thus, the less invasive, less painful collection process, combined with the possibility of retrieving a considerable amount of mesenchymal cells (enough to avoid extensive in vitro expansion), generate an effective clinical potential for cells derived from adipose tissue, compared to other methodologies.

In this way, adipose tissue has become a considerably attractive source of MSCs in surgical and regenerative medicine for cellular therapy procedures – especially in surgical and cosmetic dermatology – because it can be obtained in great amounts from a material that would otherwise be discarded in liposuction surgeries<sup>28</sup>.

This article's objective is to describe the procedures for obtaining material that has been aspirated through surgical intervention, and isolating and cultivating mesenchymal cells.

## COLLECTION, ISOLATION AND CULTIVATION: SUGGESTED PROTOCOL

### Adipose tissue collection

The discarded fat from liposuction procedures is used to collect MSCs. The adipose tissue collection procedure begins with a careful study of the site to be targeted. Photographic documentation and marking of donor areas must then be carried out. According to Jurgens and colleagues<sup>29</sup> and Almeida and others<sup>30</sup>, the donor areas that offer greater efficiency in terms of stromal vascular fraction cells are located in the trunk, followed by the limbs. In practice, however, the most frequent donor areas are the outer thigh, hips and abdomen regions.

In preparation for the lipoaspirate collection, the asepsis and antiseptics of the donor region is carried out, followed by the application of tumescent anesthesia (180 ml 0.9% saline solution, 10 ml 2% lidocaine hydrochloride without vasoconstrictor, 10 ml 8.4% sodium bicarbonate solution and 0.4 ml Epinephrine diluted to 1/1,000)<sup>31,32</sup>.

After accomplishment of a small anesthetic area in the donor area with 2% lidocaine, an incision with blade 11 is carried out and a 3 mm liposuction cannula with three orifices in the extremity, connected to 10 ml syringes, is inserted.

Around 30 ml of fat are manually aspirated under low pressure (atraumatic). If necessary, the closure is carried out with a suture (simple stitch with 6-0 mono nylon thread) at the site of introduction of the cannula, after massaging the area in order to drain fluids and blood.

The syringes containing the material collected are cleansed two or three times with sterile saline solution, sealed and left to decant for 30 minutes. Afterwards, the supernatant liquid is discarded, and the precipitate is obtained (Figure 1). The syringes containing the remaining fat are sealed and sent to the laboratory for processing.

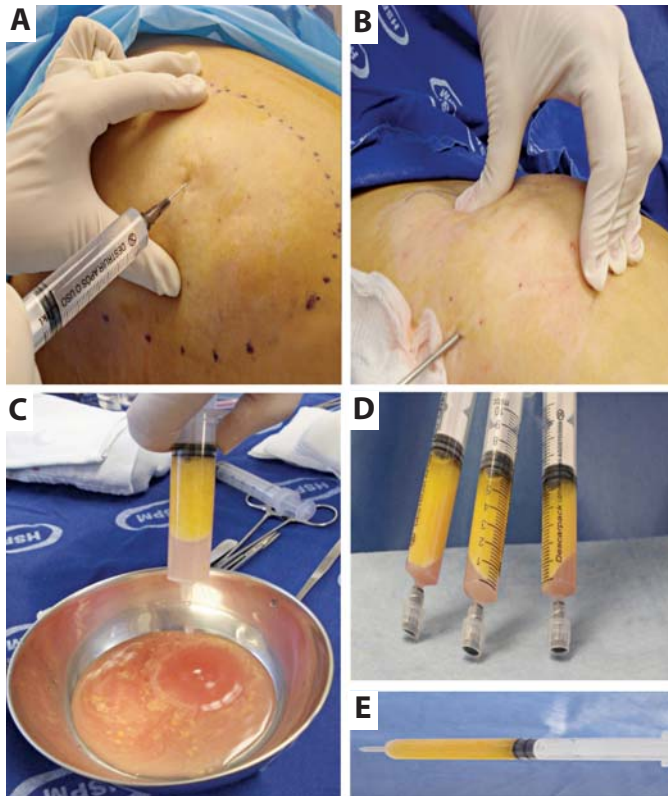
### Digestion of the adipose tissue, isolation and cultivation of mesenchymal cells

Several authors have described different techniques for this phase of the procedure<sup>24,26,33</sup>. Rodbell and others<sup>33</sup> introduced the initial method to isolate cells from adipose tissue in laboratory animals. The protocol was later adapted and used with success in human adipose tissue samples<sup>24,26</sup>.

The most commonly used methodology for isolating MSCs from lipoaspirates was first published by Zuk and colleagues<sup>24</sup>, who describe adipose tissue aspiration, digestion, cultivation and characterization of mesenchymal cells processes. The method described below is based on the Zuk technique.

#### Reagents used

- Phosphate buffered saline (PBS)
- Collagenase IV (SIGMA® Sao Paulo, Brasil)
- Cell culture medium DMEM
- Characterized fetal bovine serum (FBS, Hyclone®)
- Amikacin sulphate (Klebicil, Greenpharma®)
- Amphotericin B (Invitrogen®)



**Figure 1:** Liposuction-based adipose tissue collection procedure photomacroscopy: **A.** Tumescent anesthesia; **B.** Lipoaspirate collection; **C.** Cleansing of the fat with saline solution; **D.** Decanted material; **E.** Resulting fat

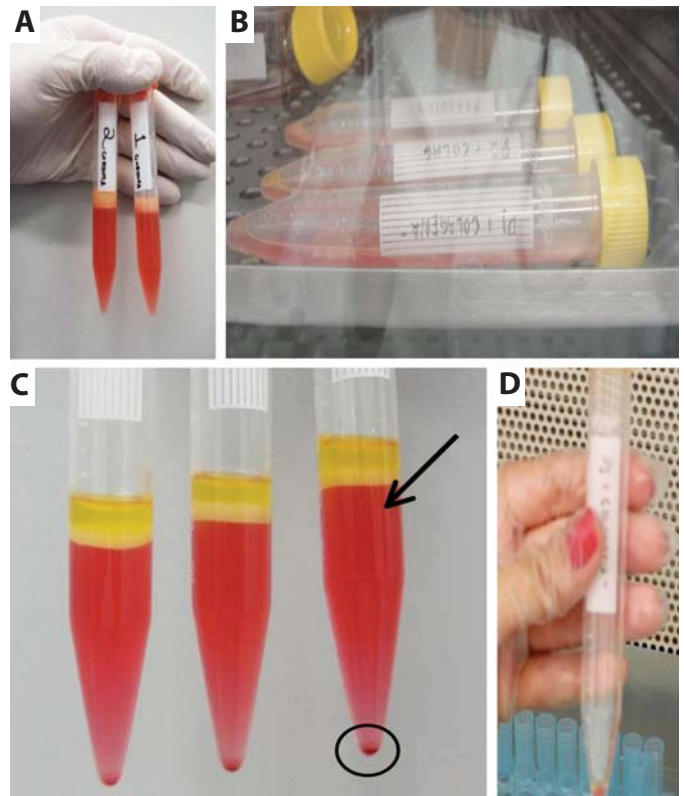
- Substitute of trypsin TrypLE™ express (Invitrogen®)
- Dimethyl sulphoxide (DMSO, Sigma®)

#### Instruments and equipment used

- 60 mm cell culture dish (TPP®)
- 15 ml and 50 ml conical tubes (TPP®)
- Pipettes (Eppendorf®)
- Pipette tips (TPP®)
- Centrifuge (Baby I centrifuge mod. 206, Fanem®)
- Laminar flow chamber (Trox technik®)
- Incubator (Forma Scientific®)
- Inverted microscope (Leica DM IRB®)
- Mr. Frosty (Nalgene®)
- Neubauer Chamber (Boeco®, Germany)

#### Procedure

All laboratory procedures are carried out in a laminar flow chamber under sterile conditions (Figure 2). The lipoaspirated adipose tissue should be successively cleansed in PBS solution so that MSCs can be obtained. After the cleansing process, the material undergoes an enzymatic digestion process with collagenase (0.010 g/ml of collagenase IV) in culture medium DMEM, supplemented with 83.4 µg/ml of amikacin sulphate and 1% amphotericin B. The collagenase solution with the adi-

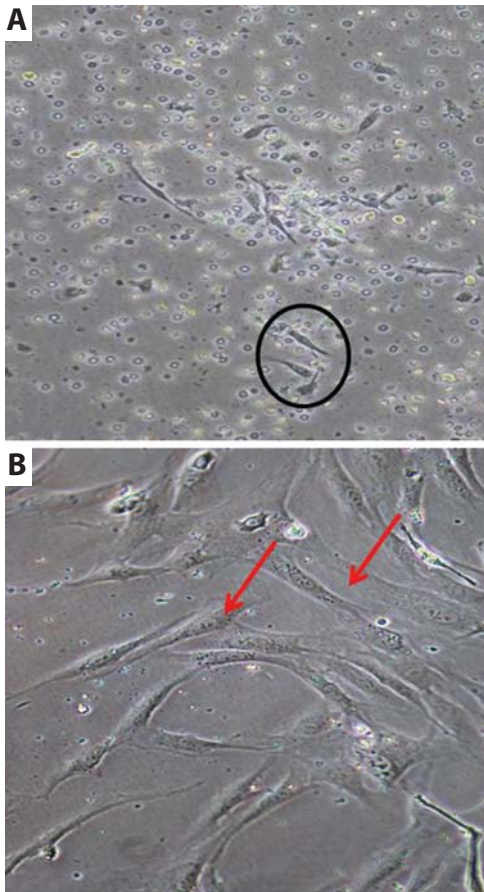


**Figure 2:** Cellular culture protocol photomacroscopy: **A.** Lipoaspirate with collagenase for cellular digestion; **B.** Cellular digestion; Figures C and D illustrate the formation of supernatant fluid (arrow) and the pellet (circle) containing the cells

pose tissue must then be kept in an incubator at 37°C, with 5% CO<sub>2</sub> for 3 hours, under controlled humidity.

After the enzymatic incubation period, the resulting solution is cleansed in culture medium supplemented with 10% FBS and antibiotic, centrifuged at 300 g for 5 minutes, and the supernatant fluid is discarded (adipose fraction). The resulting precipitate is then re-suspended in the culture medium mentioned above, and is subsequently seeded in 60 mm dishes and kept in an incubator at controlled temperature (37°C) and CO<sub>2</sub> atmosphere (5% in air).

After the first 48 hours, the culture medium is changed every other day, with the changes taking place until the cells reach a 70% confluence, then being transferred to plates. These procedures include the cellular trypsinization – the incubation of the cultivation dish with the trypsin enzyme substitute for about 10 minutes, and the inactivation of the process with half DMEM, supplemented with FBS. At this point the cells are transferred to the 15 ml capacity centrifuge's conical tubes and centrifuged at 300 g for 8 minutes; the supernatant fluid is discarded, the precipitate re-suspended, the concentration is estimated through the Neubauer Chamber, and the cells transferred again to plates. The *in vitro* culture must be followed up and documented with photographs taken using an inverted microscope (Figure 3).



**Figure 3:**  
Photomicroscopy  
of mesenchymal  
stem cells derived  
from human  
adipose tissue: A.  
Cells cultured for  
one day (circle); B.  
Cells cultured for  
four days (arrow).  
Medium: DMEM:  
A = 500  $\mu\text{m}$ ;  
B = 100  $\mu\text{m}$

The preservation and maintenance of the cellular culture is achieved through the freezing process, carried out with the trypsinization stage, as previously described: the supernatant fluid is removed while the resulting precipitate is re-suspended in a previously prepared medium for freezing (DMEM supplemented with 10% of FBS, 10% of DMSO and antibiotic) in a concentration of approximately  $1 \times 10^6$  cells/ml, distributed in cryotubes. The cryotubes are then allocated in devices specific

for freezing (Mr. Frosty) which allow a freezing curve of  $-1^\circ\text{C}$  per minute when subjected to a temperature of  $-80^\circ\text{C}$ . The device containing the cryotubes is kept in that state for 4 to 24 hours, and is then transferred to a liquid nitrogen tank, where it remains cryopreserved and stored indefinitely.

### PERSPECTIVES/OUTLOOK

Cutaneous aging is a challenge for medicine. Plastic surgeons and dermatologists are constantly required to solve problems such as filling wrinkles and deep creases. Therefore several studies are being conducted on mesenchymal cells originally from aspirated adipose tissue. Not only are they easier to obtain than other cells, but there are good regeneration outcomes in the site where these cells were applied under cellular therapy protocols<sup>6</sup>. Additionally, some clinical trials demonstrate that these cells differentiate into cells of the resident tissue<sup>34</sup>, even though it is currently believed that a possible trophic factor for these cells is the increased tissular regeneration that occurs after injuries<sup>35</sup>.

According to Shiffman and Mirrafati<sup>36</sup>, the success of the procedure depends directly on the collection, clean-up and material re-injection techniques. However, a protocol defining the best way of processing fat for a long-term graft, independently of the injected area, has not yet been standardized<sup>37</sup>.

The combination of MSCs and fat grafts aimed at increasing breast size has recently been shown to promote satisfactory results, without serious complications<sup>38</sup>, for it offers the possibility of a long-term filling<sup>39</sup>; the adipocytes' survival is one of the main factors directly interfering in the success of the grafts<sup>24,27,37</sup>.

According to Radovan Borojevic<sup>41</sup>, MSC-based cellular therapies are increasingly used in reparative surgeries, especially to repair aesthetic defects such as acne scars, rhytids, and cutaneous aging, among others. It is important to highlight that therapies that use stem cells for rejuvenation do not prevent the natural aging process. Nevertheless, cellular therapy could make it more gradual, resulting in a better quality of life regarding the human body's functionality and aesthetics. ●

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