

# Human adipose-derived stem cells: definition, isolation, tissue-engineering applications

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## Abstract

Recent researches have demonstrated that the most effective repair system of the body is represented by stem cells – unspecialized cells, capable of self-renewal through successive mitoses, which have also the ability to transform into different cell types through differentiation. The discovery of adult stem cells represented an important step in regenerative medicine because they no longer raises ethical or legal issues and are more accessible. Only in 2002, stem cells isolated from adipose tissue were described as multipotent stem cells. Adipose tissue stem cells benefits in tissue engineering and regenerative medicine are numerous. Development of adipose tissue engineering techniques offers a great potential in surpassing the existing limits faced by the classical approaches used in plastic and reconstructive surgery. Adipose tissue engineering clinical applications are wide and varied, including reconstructive, corrective and cosmetic procedures. Nowadays, adipose tissue engineering is a fast developing field, both in terms of fundamental researches and medical applications, addressing issues related to current clinical pathology or trauma management of soft tissue injuries in different body locations.

**Keywords:** stem cell, adipose tissue, tissue engineering, scaffold.

**Adult stem cells** are undifferentiated cells, found in the body after birth, that divide to replace dying cells and to regenerate damaged tissues. There are some authors who preferred the term **somatic stem cells**, as adult stem cells are found in both adults and children [1]. Somatic stem cells have the ability to divide and self-renew indefinitely in undifferentiated state, being able to generate all cell types of the organ from which they originate, even with the potential to regenerate that organ. Somatic stem cells have been isolated from various tissues of the adult body, the umbilical cord and other non-embryonic sources.

## ☐ Stem cells from the adipose tissue

White adipose tissue of the adult human body is a connective tissue derived from embryonic mesoderm, consisting of a "supportive" stroma that contains a heterogeneous population of cells like adipocytes (50–70%), preadipocytes, smooth muscle cells, endothelial cells, mast cells, fibroblasts and a variety of immune cells. The presence of the adipocyte progenitor cells in the white adipose tissue may explain their great turnover in fat deposits from all the locations in adult subjects. Approximately 10% of the entire adipocyte population is renewed annually by two antagonistic processes: "old" cells death and "young" cells adipogenesis [2]. The potential to produce new mature adipocytes from their precursors continues throughout life.

The concept of **stromal vascular fraction (SVF)** refers to a cell population resulted by white adipose tissue manipulating, including homogenization and enzymatic

digestion, differential centrifugation in order to remove the differentiated adipocytes, erythrocyte lysis and wash [3]. SVF fraction of white adipose tissue contains mesenchymal stem cells, endothelial precursor cells, preadipocytes, anti-inflammatory M2 macrophages and regulatory T-cells [4, 5]. Because in the adult organism the adipocyte progenitor cells were found in the SVF fraction of white adipose tissue, SVF cells were considered primary preadipocytes. Most commonly, SVF fraction is obtained nowadays by lipoaspiration, which is why this fraction is commonly called **processed lipoaspirate**, and the cell components are called **SVF cells** or **processed lipoaspirate cells** [6].

The efficiency of the SVF cells isolation process depends on the nature of the white adipose tissue deposits and the donor general condition (age, obesity). For example, SVF cells from visceral deposits are more prone to apoptosis and, therefore, less proliferative than the same cells isolated from the subcutaneous deposits [7].

It has been shown that SVF fraction cells can be grown in special conditions, when there is a purifying process of the cells within the culture medium, with enhancement in some cells resembling the mesenchymal stem cells. After 2001, it has been demonstrated that fat tissue is one of the richest sources in mesenchymal stem cells. Compared to bone marrow, 1 g of adipose tissue contains 500 times more pluripotent cells than 1 g of bone marrow aspirate [8].

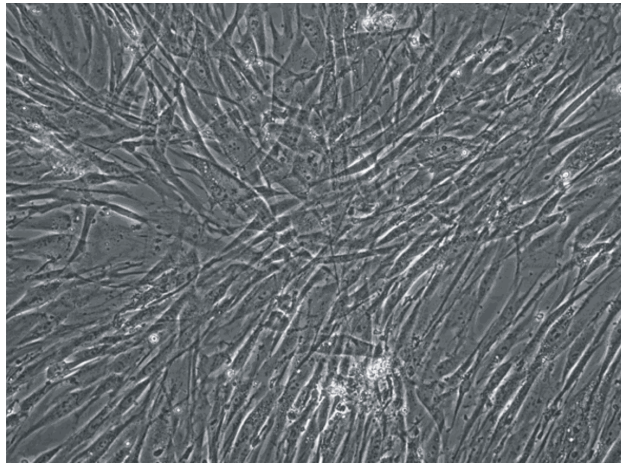
Rapid expansion of researches on white adipose tissue stem cells often generated conflicting conclusions and even inconsistency in their definition. There are some studies in the literature on unsorted SVF cell populations

regarded as white adipose tissue stem cells [9, 10], studies that define white adipose tissue stem cells as a functionally distinct subpopulation of SVF cells [11–13], or researches in which the authors do not stated clear whether the investigations are carried out throughout the entire SVF fraction or only a subpopulation of it. Furthermore, SVF fraction was analyzed as freshly prepared cells [14], or after several passages [11, 15], in some instances the culture conditions being different [16].

In the literature, there is a lot of confusion concerning the nomenclature of multipotent stem cells from adipose tissue stroma. Madonna R *et al.* [17] proposed the term of adipose tissue-derived stromal cells (ADSC) for the cellular population adherent to plastic, including here the vascular cells (pericytes and endothelial progenitors), progenitor cells for adipocytes (preadipocytes) and multivalent mesenchymal stem cells, besides circulating

blood cells, fibroblasts, endothelial cells, smooth muscle cells and immune cells (macrophages and lymphocytes) [3, 17].

To isolate ADSC, adipose tissue is minced and washed with phosphate buffered saline. Tissue fragments are incubated with collagenase and the digest is centrifuged, with separation into floating mature adipocyte population and SVF fraction sediment. All the non-adherent cells are removed after 24 hours of plating in standard medium (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal calf serum, Penicillin 100 U/mL and Streptomycin sulfate 100 mg/mL. The heterogeneous population of ADSC (Figure 1) represents the primary step to obtain adipose tissue stem cells (ASC), which includes only mesenchymal stem cells and vascular/adipocyte progenitor cells (Figure 2) [17].

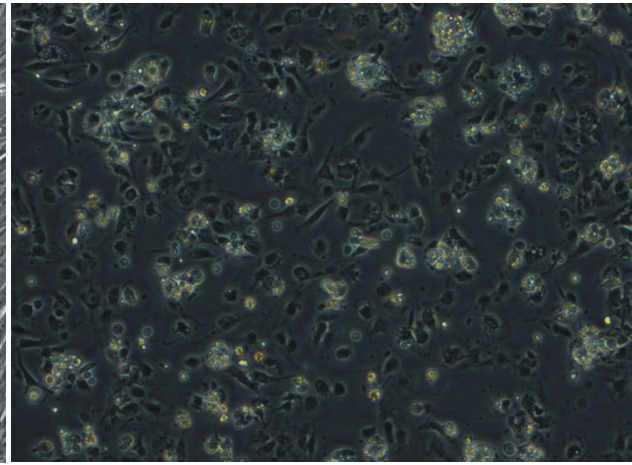


**Figure 1 – Primary ADSC cell culture, 10 days after seeding, ×10 magnification, inverted phase-contrast microscope (original picture).**

One important achievement in characterizing adipose tissue stem cells was the defining of surface markers, or cluster of differentiation (CD) antigens, especially compared with bone marrow mesenchymal stem cells and SVF cells from adipose tissue. Several investigator groups have independently examined the adipose tissue stem cells surface immunophenotype, noting different expressions depending on the passage number or the adherence to plastic. ASC express themselves specific receptor and adhesion molecules and enzymes, proteins of the extracellular matrix, cytoskeleton and stromal cell phenotype associated proteins (Table 1).

**Table 1 – Immunophenotype of human ASC in passage >2 [3]**

Antigen (Ag) category	Surface-positive antigens on ASC	Surface-negative antigens on ASC
Adhesion molecules	CD: 9, 29, 49, 54, 105, 166	CD: 11b, 18, 50, 56, 62, 104
Receptor molecules	CD: 44, 71	CD16
Enzymes	CD: 10, 13, 73	
Extracellular matrix molecules	CD90, CD146, collagen types I and III, osteopontin, osteonectin	
Cytoskeleton	α-Smooth muscle actin, vimentin	
Hematopoietic		CD: 14, 31, 45
Complement cascade	CD: 55, 59	



**Figure 2 – Undifferentiated stem cells derived from adipose tissue (ASC), ×10 magnification (original picture).**

Antigen (Ag) category	Surface-positive antigens on ASC	Surface-negative antigens on ASC
Histocompatibility antigen	HLA-ABC	HLA-DR
Stem cells	CD34, ABCG2	
Stromal	CD: 29, 44, 63, 90, 166	

ASC surface immunophenotype is similar to that of bone marrow mesenchymal stem cells [18], and cells derived from skeletal muscle [19]. Direct comparison between ASC and mesenchymal stem cell phenotype gives more than 90% identity [6]. However, there are some differences in the expression of ASC and mesenchymal stem cells surface antigens. Major difference occurs in the CD34 glycoprotein that does not appear on the surface of mesenchymal stem cells, but it is present in human ASC in early passages [18].

Stromal cells associated markers (CD13, CD29, CD44, CD63, CD73, CD90 and CD166) are initially weakly expressed on SVF cells, but this expression level significantly increases with successive passages [11]. CD34 marker, associated with stem cells, has a peak in the raw fraction of SVF cells and decreases during the passages. Aldehyde dehydrogenase and ABCG2 transport protein (multidrug-resistance transport protein) were both identified and characterized on hematopoietic stem cells, as well as expressed by the SVF and ASC at detectable levels.

Associated endothelial cell markers [CD31, CD144 or VE-cadherin, VEGFR2 (vascular endothelial growth factor receptor 2) and von Willebrand factor] are expressed on SVF cells surfaces and do not significantly adjust during passages [11].

Many studies have provided both *in vitro* and *in vivo* evidences on ASC multipotency [3, 7, 20]. Stem cell population derived from human adipose tissue digested with collagenase – named stromal vascular fraction (SVF) – suffers differentiation in multiple cell types, such as adipose tissue, cartilage, bone [21–23], skeletal muscle [24], neural cells [25, 26], endothelial cells [27, 28], cardiomyocytes [29, 30] and smooth muscle cells [12] (Table 2).

**Table 2 – Differentiation potential of the ASC cells [3]**

Cell lineage	Inductive factors
Adipocyte	Dexamethasone, isobutyl methylxanthine, indomethacin, insulin, thiazolidinedione
Chondrocyte	Ascorbic acid, BMP-6 ( <i>bone morphogenetic protein 6</i> ), dexamethasone, insulin, TGF- $\beta$ ( <i>transforming growth factor-<math>\beta</math></i> )
Osteoblast	Ascorbic acid, BMP-2 ( <i>bone morphogenetic protein 2</i> ), dexamethasone, 1,25-dihydroxy vitamin D <sub>3</sub>
Myocyte	Dexamethasone, horse serum
Cardiomyocyte	Transferrin, IL-3, IL-6, VEGF (growth factor)
Endothelial	Proprietary medium: EGM-2-MV containing ascorbate, EGF ( <i>epidermal growth factor</i> ), bFGF ( <i>basic fibroblast growth factor</i> ), hydrocortisone
Neuronal-like	Butylated hydroxyanisole, valproic acid, insulin

ASC multipotency analysis is based on morphology or typical marker expression for distinct differentiated cell types.

After demonstrating for the first time the presence of stem cells in the adipose tissue, Zuk's group generally thought that the ASC, besides the multiple mesodermal potential, have also ectodermal and endodermal differentiation potential, considering them as pluripotent stem cells [6, 8, 20].

Although, there are studies that have been reported *in vivo* and *in vitro* immunoregulatory properties for the ADSC. In contrast to SVF cells freshly isolated, the cultures of human adipose stromal cells beyond passage 1 presents a reduced level of surface expression for histocompatibility antigens and does not stimulate proliferation by allogeneic T-cells [31, 32]. If these results confirm, ADSC isolated from healthy allogeneic donors, cultured *in vitro*, will represent an exceptional stem cell source for therapeutic use in older patients [28, 33], with malignancy or obese [34], which do not allow to obtain a sufficient amount of functional ADSC, as an alternative to the autologous cells.

### ☞ Clinical applications of stem cell therapy in plastic and reconstructive surgery of the soft tissues – Tissue-engineering strategies

Reconstruction of soft tissues defects is still a significant problem because it not has been found yet an ideal filler for the correction of congenital deformities, large defects due to cancer excisions or injuries resulting from major trauma. Mature adipose tissue was used as

autologous graft in reconstruction of soft tissue defects for more than 100 years, and is still used today for this purpose due to lack of better alternatives, although the results are mediocre and unpredictable [35]. Transplants are largely resorbed gradually, adipose tissue grafts being replaced by fibrous tissue and fat cysts [36]. Poor fat autotransplant outcomes are considered to be due to decreased adipocytes tolerance to ischemia, low rate of revascularization, and therefore low rate of graft survival [37]. Adipose tissue is not only highly vascularized, with a fine capillary network that surrounds each adipocyte, but also has its own angiogenic properties. In addition, adipose tissue is highly innervated, the autonomic nervous system being responsible for the modulation of properties at cellular and molecular level. Some authors [38, 39] reported results who suggest diffuse infiltration of autologous fat with multiple passes and placement of very small aliquots at every pass, trying to separate the grafts from each other, so that each graft will be in contact with the host tissue on a greater area instead of adjacent grafts, thus creating a larger contact area between the graft and host-tissue where the nutrient vessels are found. In addition, the described method further stabilized the fat graft, preventing its migration and determines a uniform appearance of the treated area [39].

Development of adipose tissue engineering (ATE) techniques offers a great potential in surpassing the existing limits faced by the classical approaches used in plastic and reconstructive surgery. ATE clinical applications are wide and varied, including reconstructive, corrective and cosmetic procedures [40, 41].

Today, adipose tissue represents an ideal source of autologous cells for ATE strategies due to its unique expandability and accessibility. Adipose tissue can be easily obtained and in large amounts using liposuction techniques. Recent results have shown that stem cells derived from the stromal-vascular fraction of adipose tissue have a great therapeutic potential for further tissue engineering applications and cell-based therapies [42].

Nowadays, ATE is a fast developing field, both in terms of fundamental researches and medical applications, addressing issues related to current clinical pathology or trauma management of soft tissue injuries in different body locations. Soft tissue defects influence patients not only aesthetically and emotionally, but often are accompanied by impairment of those structures [43].

The general principles of tissue engineering strategies incorporate a combination of three factors: (1) living cells that are embedded in the site defect, (2) a three-dimensional (3D) protection structure (scaffold) of cells in terms of structural, functional and mechanical features, and (3) creation of a microenvironment to provide additional factors that will finally promote growth and formation of new tissue. As the new tissue is formed, the biodegradable scaffold structure must be replaced [42].

Schematically, in a tissue engineering procedure cell cultures are seeded on a biodegradable, natural or synthetic support (scaffold) in order to form a living 3D structure, structure that guides the organization, growth and differentiation of cells in the presence of appropriate growth factors. Eventually, the newly formed structure where the cells start to produce their own ECM and biomaterials used were degraded, is permanently implanted

at the patient's wounded area, thus allowing healing of the lesion.

### ❏ Cellular component of an ATE construction

Four possible roles of ASC in reparative medicine were partially confirmed by preclinical studies [44–46]. First, ASC can differentiate into adipocytes and contribute to the regeneration of the adipose tissue. Second, ASC can differentiate into endothelial cells and probably into vascular mural cells [16, 44, 46], leading to the promotion of angiogenesis and graft survival. The third role derives from the fact that ASC release angiogenic growth factors as a response to hypoxia [47] that influences the host tissue. The last role derives from the fact that ASC survive as original ASC [45].

White adipose tissue is the optimal source for the ASC isolation process. Padoin AV *et al.* [48] investigated the influence of the site of harvest on cell density. They have demonstrated that the lower abdomen and the inner aspect of the upper thigh are the best sites for harvesting adipose tissue, leading to the largest concentration of adult stem cells.

Excision and liposuction are the two competing methods of fat harvest for stem cell isolation. Studies done by von Heimburg D *et al.* [49] showed that the amount of stem cells isolated from the liposuction material is higher than the amount of cells derived from the excised adipose tissue. Overnight storage for 24 hours leads to a significant decrease of the stem cells count in the excised tissue, but not in the liposuction material. The increased number of cells isolated from the liposuction material proves that extraction by suction does not affect the stromal cellular fraction of adipose tissue. When isolation is not performed immediately after surgery, liposuction clearly represents the best alternative for adult stem cells isolation [49].

Although there are several methods for isolation of progenitor cells from the adipose aspirate, all of them go through a few common steps: removal of the hematopoietic cells, collagenase digestion and centrifugation of the digest, which allow for separation of stromal vascular fraction (SVF) which sediments. The heterogeneous SVF fraction contains, together with total differentiated cells, a number of progenitor cells. This population of SVF cells is phenotypically similar to the human mesenchymal stem cells and expresses some CD antigens identically with the mesenchymal stem cells derived from the bone marrow, but presents also a unique profile of CD markers.

### ❏ Scaffold structures for ATE construction

In the adipose tissue, extracellular matrix provides additional structural support, tensile strength, sites of attachment to cellular surface receptors and a source for signaling factors that regulate a variety of essential processes for the tissue as angiogenesis, cellular migration, proliferation, differentiation, and the immune response.

An ideal scaffold structure must accomplish the extracellular matrix roles for the cells with who is expected to form a tissue engineering construction, and to promote the repair/regeneration of damaged tissue.

Factors that govern the scaffold structure design are particularly complex, including details regarding matrix architecture, pore size and morphology, mechanical properties relative to porosity, surface characteristics and the degradation products nature. Thus, a scaffold structure should provide enough mechanical strength and stiffness to cope with the lesion contraction forces and later the tissue remodeling. In addition, a scaffold structure had to be biocompatible, to promote initial cell attachment and subsequent their migration through the matrix, to increase mass metabolites transfer and, finally, to provide sufficient space for the newly formed tissue matrix remodeling and vascular development. Scaffold materials had to be biodegradable and, during *in vitro* and/or *in vivo* remodeling process, the degradation kinetics should be enough slow to maintain structural integrity and the matrix mechanical properties. Dimensions and shape of the tissue engineering construction can be customized to every individual patient. Instead, it is imperative that scaffold structures are prepared in a reproducible way, flexible toward the presence of some biological components (cells, growth factors) in different applications [50].

Many biomaterials have been investigated in order to be used in ATE construction, both natural and synthetic polymers. The advantages of synthetic polymers rely on the technical possibility of having mechanical and chemical properties and also degradability adequate to the ATE application characteristics. Polymers like polylactic acid (PLA), poly-glycolic acid (PGA) and copolymer poly-lactic-co-glycolic acid (PLGA) suffer disintegration by acid hydrolysis and their degradability can be controlled by changing the molecular weight, crystallinity and monomer ratio [51]. PLA and PGA polymers are used predominantly in obtaining meshes, scaffold structures and/or grafts in ATE [52–54].

Natural polymers chosen for ATE supports are either compounds of the native ECM or are present in other biological systems. The advantages of natural polymers are biocompatibility, hydrophilic character, mechanical and biological properties consistent with *in vivo* features. Most common natural polymers used in ATE studies are collagen [42, 55], hyaluronan [56, 57], fibrin [58], gelatin [59], adipose tissue-derived ECM [60], decellularized human placenta [61], Matrigel [62], etc.

### ❏ Adipose tissue engineering techniques

There are many strategies for growing human adipose tissue stem cells in order to obtain an ATE construction [1]. Classic strategy uses SVF cells isolated by enzymatic digestion from the adipose aspirates with the aim to regenerate tissues, scaffold-guided. SVF cells are grown on absorbable polymeric structures and transplanted *in vivo*, for example in the breast area of a patient to fill a defect at this level. Ideally, when the scaffold structure is remodeled or is reabsorbed, preadipocytes grow, suffer differentiation and, finally, a new mature adipose tissue is created [63].

The second type of strategy uses an injectable composite system, consisting of biodegradable “microcarrier” pearls combined with a special medium that release hydrogel. Preadipocytes are cultured *in vitro* on this porous gelatin



pearls. This system represents a minimally invasive implant that will stimulate adipose cell regeneration in the host and will fill the soft tissue defect after *in vivo* injection [64].

Masuda T *et al.* [44] proposed another strategy for soft tissue enlargement based upon transplantation of a mixture of fragments of omentum – highly vascularized tissue rich in fat tissue and preadipocytes.

Other ATE methods are based on the use of acellular devices that produce *de novo* adipogenesis. An appropriate stimulus, applied *in vivo*, induces preadipocytes migration, and further proliferation and differentiation into mature adipocytes. The process was demonstrated using subcutaneous injections with Matrigel (a collagen-based gel, commercially available) and bFGF [65].

## ☞ Conclusions and future perspectives

Nowadays, regenerative medicine uses patient's own cells to regenerate a particular tissue. Tissue regeneration is done by using autologous stem cells that have the potential to differentiate into a variety of cell types. Adipose tissue is one of the richest sources of stem cells. These cells, derived from stromal vascular fraction, can differentiate into multiple adult cell types like adipose tissue, bone, cartilage, muscle and even neural cells. Promising results indicate that adipose tissue stem cells have therapeutic potential for tissue engineering applications and cell-based therapies. Further studies are needed to assess the safety of their use in humans.

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