Concise Review: Adipose-Derived Stromal Vascular Fraction Cells and Platelet-Rich Plasma: Basic and Clinical Implications for Tissue Engineering Therapies in Regenerative Surgery

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ABSTRACT

Cell-based therapy and regenerative medicine offer a paradigm shift in regard to various diseases causing loss of substance or volume and tissue or organ damage. Recently, many authors have focused their attention on mesenchymal stem cells for their capacity to differentiate into many cell lineages. The most widely studied types are bone marrow mesenchymal stem cells and adipose-derived stem cells (ADSCs), which display similar results. Based on the literature, we believe that the ADSCs offer advantages because of lower morbidity during the harvesting procedure. Additionally, platelet-rich plasma can be used in this field for its ability to stimulate tissue regeneration. The aims of this article are to describe ADSC preparation and isolation procedures, preparation of platelet-rich plasma, and the application of ADSCs in regenerative plastic surgery. We also discuss the mechanisms and future role of ADSCs in cell-based therapy and tissue engineering.

INTRODUCTION

In recent years, many scientists have shown the existence of cells in the adult body that are capable of repairing and regenerating damaged tissues. Adipose tissue is a multifunctional organ that contains various cellular types, such as mature adipocytes and the stromal vascular fraction (SVF). The adipose SVF provides a rich source of adipose-derived stem cells (ADSCs) and can easily be isolated from human adipose tissue, representing a viable alternative to bone marrow mesenchymal stem cells (BM-MSCs) [2]. Moreover, these cells can be added onto a scaffold, such as purified adipose tissue or alloplastic material, which stimulates the long-term cell retention and subsequent colonization. These techniques can be performed to treat soft tissue defects, scars, and burn injury and to regenerate various damaged tissues.

Platelet-rich plasma is a concentration of autologous human platelets in a small volume of plasma, containing at least seven major growth factors (including platelet-derived growth factors [PDGFs], basic fibroblast growth factor [bFGF], vascular endothelial growth factor [VEGF], insulin-like growth factor-1 [IGF-1], and transforming growth factor-β [TGF-β]) released by actively degranulated platelets and known to facilitate the wound-healing process. Consequently, platelet-rich plasma (PRP) is currently used in the field of the regenerative medicine because of its ability to stimulate the tissue regeneration. The aims of this article are (a) to describe the isolation procedures, molecular characterization, and differentiation capacity of ADSCs; (b) to describe the preparation of PRP and methodological changes to the preparation of PRP in the literature; and (c) to discuss the clinical application of ADSCs and PRP in regenerative plastic surgery.

PREPARATION AND MOLECULAR CHARACTERIZATION OF ADIPOSE-DERIVED STROMAL VASCULAR FRACTION CELLS

Freshly isolated SVF cells are a heterogeneous mixture of endothelial cells, smooth muscle cells, pericytes, fibroblasts, mast cells, and preadipocytes. Culture of these cells under standard conditions eventually (within the first few passages) results in the appearance of a relatively homogeneous population of mesodermal or mesenchymal cells [2] that were first named processed lipoaspirate cells but are now more commonly called ADSCs (or ASCs, which can be confused with ASCs for adult stem cells).
The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) has recently proposed a minimal set of four criteria to define human mesenchymal stem cells (MSCs) [3]:

1. MSCs are plastic-adherent when maintained under standard culture conditions.
2. MSCs have the capacity for osteogenic, adipogenic, and chondrogenic differentiation.
3. MSCs express CD73, CD90, and CD105.
4. MSCs lack expression of the hematopoietic lineage markers c-kit, CD14, CD11b, CD34, CD45, CD19, CD79, and human leukocyte antigen-DR.

The most commonly studied MSCs are BM-MSCs and ADSCs. The ADSCs meet the majority of the ISCT’s criteria for MSCs; however, Lin et al. [4] have found that ADSCs exist as CD34+ CD31− CD104b− smooth muscle actin− cells in the capillary and in the adventitia of larger vessels in vivo. In the capillary, these cells coexist with pericytes and endothelial cells, both of which are possibly related to ADSCs. In the larger vessels, these ADSCs exist as specialized fibroblasts (having stem cell properties) in the adventitia.

Corselli et al. [5] previously demonstrated that human pericytes, which encircle capillaries and microvessels, give rise in culture to genuine MSCs. This raised the question of whether all MSCs are derived from pericytes. Pericytes and other cells defined on differential expression of CD34, CD31, and CD146 were sorted from the stromal vascular fraction of human white adipose tissue. Besides pericytes, CD34+ CD31− CD146− CD45− cells, which reside in the outermost layer of blood vessels, the tunica adventitia, natively express MSC markers and give rise in culture to clonogenic multipotent progenitors identical to standard bone marrow-derived MSCs. Despite common MSC features and developmental properties, adventitial cells and pericytes retain distinct phenotypes and genotypes through culture. However, in the presence of growth factors involved in vascular remodeling, adventitial cells acquire a pericyte-like phenotype. In conclusion, Corselli et al. [5] demonstrated the coexistence of two separate perivascular MSC progenitors: pericytes in capillaries and microvessels and adventitial cells around larger vessels.

Factors such as donor age, adipose tissue type (white or brown) and anatomical location (subcutaneous or visceral adipose tissue), type of surgical procedure, culturing conditions, exposure to plastic, plating density, and medium formulations might influence both the proliferation rate and the differentiation capacity of these cells. For example, peroxisome prolifera-
tor-activated receptor-γ (PPAR-γ) [6] is an indicator of adipogenic potential and commitment) is expressed more by the adipose tissue samples taken from the arm [6].

In addition, there is evidence that the ability of adipose precursor cells to grow and differentiate varies among fat depots and changes with age. It has also been demonstrated that a PPAR-γ agonist aids in the induction of differentiation in cells from all depots and ages. Younger patients have increased PPAR-γ expression in all depots, whereas older patients have consistent elevated expression only in the arm and thigh depots [6]. In fact, the growth rate of ADSCs from SVF was higher in a group of patients 25–30 years old than in older patients. Superficial abdominal adipose tissue appears to be less susceptible to apoptotic stimuli [6].

Levi et al. [7] reported that subcutaneous fat depots retain markedly different osteogenic and adipogenic potentials. Osteogenesis is most robust in adipose-derived stromal cells from the flank and thigh, as compared with those from the arm and abdomen (p < .05 by all markers examined). This is accompanied by elevations in bone morphogenetic protein 4 and bone morphogenetic protein receptor 1B (p < .05 by all markers examined). The osteogenic advantage of cells from the flank and thigh is also observed when the paracrine effects of these cells are analyzed. Conversely, those cells isolated from the flank have a lesser ability to undergo adipogenic differentiation. Adipose-associated HOX genes are less expressed in flank-derived adipose-derived stromal cells. Variations exist between fat depots in terms of adipose-derived stromal cell osteogenic and adipogenic differentiation. Differences in HOX expression and bone morphogenetic protein signaling may underlie these observations. This study indicates that the choice of fat depot derivation of adipose-derived stromal cells may be an important one for future efforts in tissue engineering.

Neither the type of surgical procedure nor the anatomical site of the adipose tissue affects the total number of viable cells that can be obtained from the SVF [8]. However, since different anatomical localizations of fat tissues have their own metabolic characteristics, such as lipolytic activity, fatty acid composition, and gene expression profile, the source of subcutaneous adipose tissue grafts (abdominal-subcutaneous vs. peripheral-subcutaneous) might influence the long-term characteristics of the fat graft.

In a study by Aksu et al. [9] in which human ADSCs were isolated from superficial and deep adipose layers of the abdominoplasty specimens obtained from patients (male and female) undergoing elective surgeries, there was no significant difference in the degree of osteogenic differentiation between the ADSCs from both depots in the female. In the male, the superficial depot ADSCs differentiated faster and more efficiently than those of the deep depot. Male ADSCs from both depots differentiated more effectively than female ADSCs from both depots.

The frequency of proliferating SVF cells and the population doubling time are dependent on the surgical procedure, with some advantages for resection and tumescent liposuction compared with ultrasound-assisted liposuction [10]. In one study comparing BM-MSCs and lipoaspirate-derived ADSCs [10] from the same patient, no significant differences were observed regarding the yield of adherent stromal cells, growth kinetics, cell senescence, multilineage differentiation capacity, or gene transcription efficiency. Metabolic characteristics and fat cell viability seem not to differ when comparing standard liposuction with syringe aspiration, and no unique combination of preparation or harvesting techniques has appeared superior to date [8]. Although attachment and proliferation capacity are more pronounced in ADSCs derived from younger donors compared with older donors, the differentiation capacity is maintained with aging [8].

ADSCs have the same differentiation potential as described for BM-MSCs. However, some characteristics, such as the colony frequency and the maintenance of proliferating ability in culture, seem even to be superior in ADSCs compared with BM-MSCs [8]. The proliferation of ADSCs can be stimulated by fibroblast growth factor 2 (FGF-2) via the FGF-receptor-2 [11], by sphingosylphosphorylcholine via activation of c-Jun N-terminal kinase (JNK) [11], by platelet-derived growth factor via activation of JNK.
[12], and by oncostatin M via activation of the microtubule-associated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) and the JAK3/STAT1 pathway [13]. ADSCs do express an autocrine FGF-2 loop that maintains their self-renewal ability in vitro [14]. Since inhibition of MEK1 reduces the clonogenic potential of SVF without affecting their differentiation potential, the ERK1/2 signaling pathway seems to be involved in the FGF-2-mediated self-renewal [14]. In addition, the longevity of human ADSCs can be extended by overexpression of the catalytic subunit of the human telomerase gene [15]. ADSCs are known to secrete potent growth factors, such as VEGF, hepatocyte growth factor (HGF), and IGF-1 [16]. Tumor necrosis factor can significantly increase the secretion of VEGF, HGF, and IGF-1 from ADSCs by a p38 mitogen-activated protein kinase-dependent mechanism [16].

According to Yu’s protocol [17] for manual SVF extraction, we performed the following steps: (a) We warmed up the buffer (500 ml or more of phosphate-buffered saline [PBS] or KRB) and freshly prepared collagenase solution. (b) We prepared PBS solution with 1% bovine serum albumin, filtered the solution, and warmed it at 37°C. (c) We dispensed a volume of adipose tissue in sterile 250-ml plastic bottles (each bottle could accommodate ~100 ml of tissue). We then added an equal volume of warm PBS, agitated it to wash the tissue for ~5 minutes, and aspirated the infranatant solution. The wash was repeated three times. (d) We then added an equal volume (60–70 ml) of warm 0.1% collagenase type I (C130; Sigma-Aldrich, Milan, Italy, http://www.sigmaaldrich.com) into the bottles containing the washed adipose tissue sample and placed them in a shaking water bath at 37°C at ~75 rpm for 60 minutes. (e) Samples were then centrifuged for 10 minutes at 600g at room temperature. (f) We removed the supernatant, containing mature adipocytes and oil, and the underlying layer of collagenase solution. The pelleted cells were centrifuged at 300g for 5 minutes at room temperature in erythrocyte lysis buffer (155 mM NH4Cl, 10 mM KHCO3, and 0.1 mM EDTA) and incubated for 5 minutes at room temperature after centrifugation at 1,100 rpm for 5 minutes. The pellet was resuspended in a few microliters of growth medium and passed through a 100-mm Falcon strainer (Becton, Dickinson and Company, Sunnyvale, CA, http://www.bd.com). Counting the cellular population using a hemocytometer, we obtained ~250,000 ± 34,782 nucleated cells per milliliter of adipose tissue (12 donors were used to develop this value) [18].

**DIFFERENTIATION CAPACITY OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS**

Allocation and Differentiation

There is increasing evidence of the ability of the SVF to differentiate into cells of nonmesodermal origin, such as neurons, endocrine pancreatic cells, hepatocytes, endothelial cells, and cardiomyocytes. Accordingly, we have elected to describe this process using the term cross-differentiation (cross-germplasticomyocytes). We hypothesize that the mechanism of regeneration of the tissue is as follows: targeting of damaged areas and release of angiogenic and antiapoptotic factors, followed by formation of new vessels and oxygenation. The potential benefit of SVF supplementation could be explained by the ability of cells that exist within the SVF population [19] to secrete various growth factors that improve survival and increase vascularization [20, 21], leading to an increased survival of the graft as shown by a study on a rodent [22]. Our results documented that the SVF cell yield from the manual system was much greater than that of the automatic system.

**TRANSITION FROM ISOLATION TO MECHANISM OF REGENERATIVE PROPERTIES**

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Table 1. Cell lineages of adipose-derived stem cell differentiation

| Cell lineage | Inductive factors | Molecular and gene expression |
|--------------|------------------|-------------------------------|
| Adipocyte    | Dexamethasone, isobutyl methylxanthine, indomethacin, insulin, thiazolidinedione | Lipoprotein-lipase, αP2, PPAR-γ2, leptin, Glut4 |
| Osteoblast   | Ascorbic acid, BMP-2, dexamethasone, 1,25-dihydroxyvitamin D | Alkaline phosphatase, type 1 collagen, osteopontin, osteocalcin, bone sialoprotein, RunX-1, BMP-2, BMP-4, BMPRI, BMPRII, PThr |
| Chondrocyte  | Ascorbic acid, BMP-6, dexamethasone, insulin, TGF-β | Sulfate-proteoglycans, collagen II, collagen VI, aggrecan, PREPL |
| Neuronal-like | Butylated acid, valproic acid, insulin | Nestin, NSE, NeuN |
| Myocyte      | Dexamethasone, horse serum | MyoD1 e myf4, myf6 e miogenina |

Abbreviations: BMP, bone morphogenetic protein; BMPRI, bone morphogenetic protein receptor; PPAR, peroxisome proliferator–activated receptor; TGF, transforming growth factor.

Assessment of Adipogenic, Osteogenic, and Neurogenic Differentiation of Human ADSCs

In culture, the ADSCs can differentiate into mesenchymal and nonmesenchymal cell lineages when stimulated by inductive factors. Cell lineages are reported in Table 1. The adipogenic and osteogenic potential of ADSCs was determined as reported by Cervelli et al. in 2009 [35]. Neurogenic differentiation was induced in ADSCs and verified with anti-S100 antibody staining (NeoMarkers; Lab Vision, Fremont, CA, http://www.labvision.com).

PRP PREPARATION

The types of PRP preparation may be divided into two categories: manual methods and methods that rely on use of semiautomatic or automatic devices. Briefly, the process of preparing PRP consists of four phases: blood collection, centrifugation for platelet concentration, induction of gelation (if the PRP is to be used in gel form), and activation.

The manual preparation of PRP consisted of a slow centrifugation, which allowed the platelets to remain suspended in the plasma while the leukocytes and erythrocytes were displaced to the bottom of the tube. A rapid centrifugation can cause mechanical forces and elevate the temperature, thus inducing changes in the ultrastructure of platelets that, in turn, can initiate a partial activation, with a subsequent loss of their granular content [35]. Current systems for the preparation of platelet concentrations routinely report the use of various centrifugation rates (we used 1,100 g). After centrifugation, the buffy coat layer, consisting of platelets and white blood cells, was sequestered in a volume of 9 ml of plasma.

Anita et al. [36] reported the use of two centrifugation rates. Blood was collected into 3.8% (wt/vol) sodium citrate and centrifuged at 4,500g for 12 minutes at 4°C to obtain platelet-poor plasma (PPP) or at 460g for 8 minutes to obtain PRP. Calcium chloride was added to PPP and PRP at a final concentration of 22.8 mM. The secretion of growth factor begins with platelet activation. Our PRP protocol uses Ca$^{2+}$ to induce platelet activation and exocytosis of the α granules [35].

Standard cell separators and salvage devices can be used to produce platelet-rich plasma. These devices operate on a unit of blood and typically use continuous-flow centrifuge bowl or continuous-flow disk separation technology and both a hard (fast) and a soft (slow) spin, yielding platelet concentrations of $2 \times \times 4 \times$ baseline [37, 38]. Such devices include the CATS (Fresenius, Wilmington, DE, http://www.fmcna.com), Sequestra (Medtronic, Minneapolis, http://www.medtronic.com), Hemo-
**PRP Increases Adipose Tissue Stem Cells Number In Vitro**

As reported, PRP induced an increase of ADSC number without any morphological changes compared with control. There was a statistically significant increase, by approximately fourfold, at 4 and 6 days, when cells were preconfluent ($p < .02$). After 8 days, at confluence, there was a threefold increase of ADSC numbers in PRP cultures compared with controls. Oil Red O staining did not reveal any significant difference in intracytoplasmic lipid accumulation compared with PRP-treated and control ADSCs [35].

**Current Clinical Applications**

**PRP Therapies**

The present findings demonstrated that different concentrations of PRP have effects on ADSC proliferation. In the range from 0.2 to 0.4 ml, we observed an increase in the rate of proliferation and, more precisely, determined in vitro that the concentration of 0.4 ml was optimal; higher concentrations did not further affect the proliferation rate.

We expect that there will be new findings reported in the literature about the selection of the most appropriate regenerative methods. Indeed, there are already many publications regarding the use of PRP with/without fat graft in plastic and reconstructive surgery.

We have reported results obtained using PRP mixed with fat grafting in the treatment of chronic lower-extremity ulcers [45–47], loss of substance on the lower limbs [47], and application of the enhanced stromal vascular fraction in post-traumatic lower-extremity ulcers [18].

In a study by Crovetti et al. [48], 24 patients with chronic cutaneous ulcers were treated with a series of PRP gel treatments. Nine patients demonstrated complete wound healing. There were no adverse effects encountered, and all patients noted decreased pain [48]. Another wound study by McAleer et al. [49] involved 24 patients with 33 chronic nonhealing lower-extremity wounds. The wounds were injected with PRP every 2 weeks. Successful wound closure and epithelization were obtained in 20 wounds. The mean time for closure was 11.15 weeks [49]. These findings were particularly significant because all patients had failed previously available treatment methods.

Rozman and Bolta [50] reported the use of only PRP in skin and soft-tissue losses on the basis of its bactericidal and cell proliferation-promoting properties.

Cervelli et al. described the evaluation of the healing rate of chronic skin ulcer [45] according to the method of Kazakos et al. [51] with minor modifications. Kazakos et al. [51] performed a study to assess the benefits of using autologous PRP gel in the treatment of 59 patients affected by acute limb soft tissue wounds. The clinical end points were the healing rate and/or the time required to bring about adequate tissue regeneration to undergo reconstructive plastic surgery. That study showed that PRP gel treatment can be a valuable and effective aid in the management of acute trauma wounds.

Powell et al. [52] describe anti-inflammatory properties with reduced edema and ecchymosis associated with the autologous platelet gel in eight women after deep-plane rhytidectomy (face lifting). PRP was also shown to be effective in stopping capillary bleeding in the surgical flaps of a series of 20 patients undergoing various types of cosmetic surgery (face lift, breast size changes, or neck lifts) reported by Man et al. [53]. Guerrerosantos [54] reported the use of fat tissue without PRP in patients affected by Romberg syndrome disease and facial defects. In the facial tissue atrophy types 1 and 2, Guerrerosantos [54] reported that treatment with lipoinjection alone, based on the use of fat tissue without PRP and/or ADSC, was sufficient. In addition, Guerrerosantos et al. [55] reported some cases treated with rhitido-plasty combined with pursing plication suspension sutures and lipoinjection. They showed interesting results in the facial and cervical flaccidity and concluded that this combination of procedures provides a three-dimensional esthetic improvement in contour and volume, a reduced time of convalescence and recovery, and decreased risk for complications, especially in the facial nerves [55].

Recently, the use of fat graft with plateleterich plasma [56] and with only liposuction technique [57] have been reported in patients affected by Romberg syndrome. Platelet-rich plasma mixed with centrifuged fat tissue followed by the Coleman technique [33, 58] (for reconstructing the three-dimensional projection of the facial contour and restoring the superficial density of the facial tissues) yielded results that support the efficacy of this combination treatment. This was further confirmed on the basis of patient satisfaction.

Azzena et al. [59] hypothesized that autologous platelet-rich plasma could be used as an in vivo adipocyte delivery system to favor cell survival and to stimulate early recruitment of microcapillaries to the site of implantation, and they reported on the treatment of a patient affected by adherent scar. Azzena et al. [59] considered fat transplantation as a tissue regenerator and as a vehicle carrying its stromal fraction very rich in precursor cells and stem cells into the injection site, and indicated that this type of tissue manipulation should be performed only by well-trained surgeons in well-equipped and reliable facilities.

**ADSC Therapies**

On the basis of both in vitro experiments and preclinical studies, ADSCs have been applied to various clinical fields. In the first clinical case, autologous ADSCs were used for the regenerative treatment of widespread traumatic calvarial bone defects [21]. A 7-year-old girl with post-traumatic calvarial defects was treated with autologous cancellous iliac bone combined with autologous ADSCs, fibrin glue, and a biodegradable scaffold. Postoperative computed tomography showed new bone formation, and almost complete calvarial continuity was obtained.

The transfer of ADSCs combined with free fat has been reported to play an important role in maintaining the volume of the injected fat tissue [60]. Injection of free fat together with ADSCs isolated from the equivalent lipectomy aspirates, termed cell-assisted lipotransfer, could become an alternative to soft tissue augmentation surgery, including cosmetic breast augmentation [20]. A new aim could be the use of fresh ADSCs isolated from half of the fat tissue mixed with platelet-rich plasma and recombinated with the other half. Autologous ADSC therapy could also be used to treat fistulas in patients with Crohn’s disease.

In a pilot study of five patients with Crohn’s disease, the external openings in six of eight fistulas were closed by inoculation of the fistulas with autologous ADSCs [23]. Since that report was published, ADSCs have been also used to repair tracheomediastinal fistulas caused by cancer ablation [61].

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Stem Cells Translational Medicine
The therapeutic potential of ADSCs for wound healing can be anticipated for the treatment of chronic ulcers caused by radiation therapy [21]. Twenty patients being treated for the side effects of radiotherapy, and with severe symptoms or irreversible functional damage, received autologous ADSCs delivered via repeated hypoinvasive computer-assisted injections. The clinical outcome was systematic improvement or remission of symptoms in all patients evaluated. Although the mechanism of therapy at the molecular level is unknown, this therapeutic approach may play a pivotal role in the treatment of intractable ulcer.

In addition, ADSCs have in vivo immunosuppressive properties [62], such as have been demonstrated in a clinical trial in which acute graft versus host disease resolved completely in five of six patients, four of whom were alive, without side effects, after a median follow-up period of 40 months [63]. Finally, clinical trials of ADSCs for the treatment of both chronic heart failure and acute myocardial infarction have begun in Europe.

**CONCLUSION**

Since their isolation and characterization nearly a decade ago, adipose-derived stem cells have become one of the most popular adult stem cell populations for research in soft tissue engineering and regenerative medicine applications. Compared with other stem cell sources, ADSCs offer several advantages, including an abundant autologous source, minor invasive harvesting (liposuction), significant proliferative capacity in culture, and multilineage potential. Numerous preclinical studies have been pursued, with early clinical data appearing in the literature. This review focuses on the published clinical and preclinical data to date using ADSCs and PRP for soft tissue reconstruction, with particular attention to experimental models and methodologies. Our opinion on the future directions for making tissue regenerative therapies more effective is that ADSCs and growth factors will be used in all pathologies for autologous reconstruction of our tissue and organs. Although standardization of ADSC harvesting and processing techniques, as well as long-term results of existing clinical studies, remains to be addressed, the known biological properties of ADSCs suggest a potential role in enhancing fat graft retention and facilitating minimally invasive reconstructive treatments. Although clinical applications are being reported, well-controlled clinical studies are needed to demonstrate safety and efficacy.

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**AUTHOR CONTRIBUTIONS**

P.G.: conception and design, manuscript writing; C.D.P. and I.B.: data analysis and interpretation; M.G.S.: collection and assembly of data; A.O.: conception and design, provision of study material; V.C.: administrative support, final approval of the manuscript.

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