STEM CELLS TRANSLATIONAL MEDICINE™

TISSUE ENGINEERING AND REGENERATIVE MEDICINE

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

PIETRO GENTILE,[a] AUGUSTO ORLANDI,[b] MARIA GIOVANNA SCIOLI,[b] CAMILLA DI PASQUALI,[a] ILARIA BOCCHINI,[a] VALERIO CERVELLI[a]

Key Words. Adipose • Stromal cells • Tissue-specific stem cells • Adipose stem cells

[a]Plastic and Reconstructive Surgery Department and [b]Institute of Anatomic Pathology, Tor Vergata University, Rome, Italy

Correspondence: Pietro Gentile, M.D., San Salvatore in Lauro Place, 15, Rome, Italy. Telephone: +393388515479; Fax: +390623188426; e-mail: pietrogentile2004@libero.it

Received November 15, 2011; accepted for publication January 20, 2012; first published online in SCTM *EXPRESS* February 22, 2012.

1066-5099/2012/$20.00/0

http://dx.doi.org/10.5966/sctm.2011-0054

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. STEM CELLS TRANSLATIONAL MEDICINE *2012;1:230–236*

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 [1] 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 outmost 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 proliferator-activated receptor-γ (PPAR-γ) (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-2 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 abdomen 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 transduction 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 3–5 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 600*g* 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 300*g* for 5 minutes at room temperature in erythrocyte lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 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].

For automatic SVF extraction, the Celution system (Cytori Therapeutics, Inc., San Diego, CA, http://www.cytori.com) was used [4]. (a) Adipose tissue was introduced into the cell-processing device and subsequently washed to remove red blood cells and debris, and then enzymatically digested with collagenase type I. Upon digestion of adipose tissue and release of mononuclear cells from the adipose tissue matrix, the released cells were transferred into the centrifuge processing vessel. (b) The cells within the suspension were concentrated by short centrifugation and wash cycles. The cycles were repeated until the entire volume of input cell suspension has been processed and the cell population had been localized into the output chamber. (c) The cells were washed one final time and then suspended for use in 5 ml of Ringer's lactate solution. (d) The SVF pellet was resuspended in erythrocyte lysis buffer, and after centrifugation at 1,100 rpm for 5 minutes, the cellular population was counted using hemocytometer. The cells yield was ~50,000 ± 6,956 nucleated cells per milliliter of adipose tissue (10 donors were used to develop this value) [18].

Transition from Isolation to Mechanism of Regenerative Properties

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.

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-germ plasticity).

The transcriptional and molecular events triggering the lineage-specific mesodermal differentiation into adipocytes [23–25], myocytes [26–28], osteocytes [29, 30], and chondrocytes [29] are well known, and several reviews focus on that point. In the case of adipocyte differentiation, although several transcriptional key events regulating the differentiation of preadipocytes into mature adipocytes have been identified in the last decade, master genes committing the multipotent mesenchymal stem cells to adipoblasts are still awaiting discovery. Recently, a transcriptional coactivator with PDZ-binding motif tafazzin gene (TAZ) was identified as an early "molecular rheostat" modulating mesenchymal stem cell differentiation [31, 32]. Whereas runx-2, the key osteogenic transcription factor, triggers MSC to an osteogenic differentiation program, adipogenic differentiation is mainly promoted by PPAR. It is mainly of interest how these two transcription factors are regulated to determine these alternative cell fates. Hong and Yaffe [32] demonstrated that TAZ coactivates runx-2-dependent gene transcription and inhibits PPAR-dependent gene transcription. As a net result, osteogenic differentiation is favored. By modulating TAZ expression in cell lines, mouse embryonic fibroblasts, primary MSCs in culture, and in zebrafish in vivo, Hong and Yaffe [32] were successful in triggering osteogenic versus adipogenic differentiation. These results indicate that TAZ functions as a real molecular rheostat that allocates MSCs to either osteogenic or adipogenic differentiation. In this context, catenin signaling and Wnt3a are important mediators in reducing the osteogenic differentiation in ADSCs [33]. However, TAZ has been shown specifically only in BM-MSCs, although a recent article by Cho et al. [34] suggests that TAZ is expressed in ADSCs.

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, aP2, 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, BMPR I, BMPR II, 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; BMPR, 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.

Anitua et al. [36] reported the use of two centrifugation rates. Blood was collected into 3.8% (wt/vol) sodium citrate and centrifuged at 4,500*g* for 12 minutes at 4°C to obtain platelet-poor plasma (PPP) or at 460*g* 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× to 4× baseline [37, 38]. Such devices include the CATS (Fresenius, Wilmington, DE, http://www.fmcna.com), Sequestra (Medtronic, Minneapolis, http://www.medtronic.com), Hemonetics Cell Saver 5 (Haemonetics Corp., Braintree, MA, http://www.haemonetics.com), and others [37–39]. Many surgical procedures require the use of relatively small volumes of platelet-rich plasma [40]. Consequently, small, compact office systems have been developed that produce approximately 6 ml of platelet-rich plasma from 45–60 ml of blood [40–42]. There are many such systems, including the GPS (Biomet, Warsaw, IN, http://www.biomet.com), PCCS (Implant Innovations, Inc., Palm Beach Gardens, FL, http://www.3i-online.com), Symphony II (DePuy, Warsaw, IN, http://www.depuy.com), SmartPReP (Harvest Technologies Corp., Norwell, MA, http://www.harvesttech.com), and Magellan (Medtronic) [37, 39–42]. Although all operate on a small volume of drawn blood (45–60 ml) and on the principle of centrifugation, these systems differ widely in their ability to collect and concentrate platelets, with approximately 30%–85% of the available platelets collected and a less-than-twofold to approximately eightfold increase in the platelet concentration over baseline [37, 38].

There are several devices for the PRP preparation, such as Fibrinet (Cascade Medical Enterprises, Plymouth, U.K., http://www.cascademedical.com), Regen (Regen Lab, Le Mont-sur-Lausanne, Switzerland, http://www.regenkit.com), Plateltex (Plateltex S.R.O., Bratislava, Slovakia, http://www.plateltex.com), and Vivostat (Vivostat A/S, Borupvang, Denmark, http://www.vivostat.com). Generally, we prepared PRP according to the Cascade method, and in all cases we worked under a protocol approved by our institution's transfusion service.

In general, most systems, whether large or small volume, do not concentrate the plasma proteins of the coagulation cascade [37, 39]. The concentration of plasma protein levels above baseline can be achieved through secondary ultrafiltration, as is done with the UltraConcentrator (Interpore Cross, Irvine, CA) and the Access System (Interpore Cross), in which the buffy coat collected from a centrifugation stage is passed through hollow fibers with an effective pore size of 30 kDa. This system removes by filtration up to two-thirds of the aqueous phase; thus, the concentrations of the retained plasma proteins and formed elements are increased substantially [43].

In an interesting work, Mazzucco et al. described the different growth factor concentrations that are obtained through different devices (Fibrinet, Plateltex, and Regen) and a homemade method [44]. The PDGF-BB, TGF-β, and IGF-1 were detected in lower concentrations with the use of Fibrinet. In contrast, the Regen method showed high concentrations of TGF-β, bFGF, and IGF-1, whereas the Plateltex method showed a high level of endothelial growth factor [44].

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 epitheliazation 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 rhytidoplasty 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 platelet-rich plasma [56] and with only lipostructure 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 liposuction 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 recombined 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].

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.

Acknowledgments

The authors gratefully acknowledge Professor Jeffrey M. Gimble, Stem Cell Biology Laboratory Pennington Biomedical Research Center, Louisiana State University System, who provided comments and suggestions on the manuscript during its preparation.

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.

References

1 Katz AJ, Tholpady A, Tholpady SS et al. Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hA-DAS) cells. Stem Cells 2005;23:412–423.

2 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.

3 Dominici M, Le Blanc K, Mueller I et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315–317.

4 Lin K, Matsubara Y, Masuda Y et al. Characterization of adipose tissue-derived cells isolated with the Celution™ system. Cytotherapy 2008;10:417–426.

5 Corselli M, Chen CW, Sun B et al. The tunica adventitia of human arteries and veins as a source of mesenchymal stem cells. Stem Cells Dev 2011 [Epub ahead of print].

6 Schipper BM, Marra KG, Zhang W et al. Regional anatomic and age effects on cell function of human adipose-derived stem cells. Ann Plast Surg 2008;60:538–544.

7 Levi B, James AW, Glotzbach JP et al. Depot-specific variation in the osteogenic and adipogenic potential of human adipose-derived stromal cells. Plast Reconstr Surg 2010; 126:822–834.

8 Xu Y, Malladi P, Wagner DR et al. Adipose-derived mesenchymal cells as a potential cell source for skeletal regeneration. Curr Opin Mol Ther 2005;7:300–305.

9 Aksu AE, Rubin JP, Dudas JR et al. Role of gender and anatomical region on induction of osteogenic differentiation of human adipose-derived stem cells. Ann Plast Surg 2008;60: 306–322.

10 De Ugarte DA, Morizono K, Elbarbary A et al. Comparison of multi-lineage cells from human adipose tissue and bone marrow. Cells Tissues Organs 2003;174:101–109.

11 Jeon ES, Song HY, Kim MR et al. Sphingosylphosphorylcholine induces proliferation of human adipose tissue-derived mesenchymal stem cells via activation of JNK. J Lipid Res 2006;47:653–664.

12 Kang YJ, Jeon ES, Song HY et al. Role of c-Jun N-terminal kinase in the PDGF-induced proliferation and migration of human adipose tissue-derived mesenchymal stem cells. J Cell Biochem 2005;95:1135–1145.

13 Song HY, Jeon ES, Jung JS et al. Oncostatin M induces proliferation of human adipose tissue-derived mesenchymal stem cells. Int J Biochem Cell Biol 2005;37:2357–2365.

14 Zaragosi LE, Ailhaud G, Dani C. Autocrine fibroblast growth factor 2 signaling is critical for self-renewal of human multipotent adipose-derived stem cells. Stem Cells 2006;24: 2412–2419.

15 Jun ES, Lee TH, Cho HH et al. Expression of telomerase extends longevity and enhances differentiation in human adipose tissue-derived stromal cells. Cell Physiol Biochem 2004; 14:261–268.

16 Wang M, Crisostomo P, Herring C et al. Human progenitor cells from bone marrow or adipose tissue produce VEGF, Hgf, and IGF-1 in response to Tnf by a p38 mitogen activated protein kinase dependent mechanism. Am J Physiol Regul Integr Comp Physiol 2006;291: R880–R884.

17 Yu G, Floyd E, Wu X et al. Isolation of human adipose-derived stem cells from lipoaspirates. Methods Mol Biol 2011;702: 17–27.

18 Cervelli V, Gentile P, De Angelis B et al. Application of enhanced stromal vascular fraction and fat grafting mixed with PRP in post-traumatic lower extremity ulcers. Stem Cell Res 2011;6:103–111.

19 Rigotti G, Marchi A, Galie M et al. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: A healing process mediated by adipose-derived adult stem cells. Plast Reconstr Surg 2007;119: 1409–1422.

20 Yoshimura K, Sato K, Aoi N et al. Cell-assisted lipotransfer for cosmetic breast augmentation: Supportive use of adipose-derived stem/stromal cells. Aesthetic Plast Surg 2008; 32:48–55.

21 Lendeckel S, Jödicke A, Christophis P et al. Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: Case report. J Craniomaxillofac Surg 2004;32:370–373.

22 García-Olmo D, García-Arranz M, García LG et al. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. Dis Colon Rectum 2005;48:1416–1423.

23 Rosen ED. The molecular control of adipogenesis, with special reference to lymphatic

pathology. Ann NY Acad Sci 2002;979:143–158; discussion 188–196.

24 Schäffler A, Muller-Ladner U, Scholmerich J et al. Role of adipose tissue as an inflammatory organ in human diseases. Endocr Rev 2006;27:449–467.

25 Lane MD, Tang QQ. From multipotent stem cell to adipocyte. Birth Defects Res A Clin Mol Teratol 2005;73:476–477.

26 Chargé SB, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. Physiol Rev 2004;84:209–238.

27 Brand-Saberi B. Genetic and epigenetic control of skeletal muscle development. Ann Anat 2005;187:199–207.

28 Tajbakhsh S. Skeletal muscle stem and progenitor cells: Reconciling genetics and lineage. Exp Cell Res 2005;306:364–372.

29 Otto WR, Rao J. Tomorrow's skeleton staff: Mesenchymal stem cells and the repair of bone and cartilage. Cell Prolif 2004;37:97–110.

30 Dani C. Embryonic stem cell-derived adipogenesis. Cells Tissues Organs 1999;165: 173–180.

31 Hong JH, Hwang ES, McManus MT et al. TAZ, a transcriptional modulator of mesenchymal stem cell differentiation. Science 2005; 309:1074–1078.

32 Hong JH, Yaffe MB. TAZ: A beta-catenin-like molecule that regulates mesenchymal stem cell differentiation. Cell Cycle 2006;5: 176–179.

33 Coleman SR. Facial recontouring with lipostructure. Clin Plast Surg 1997;24:347.

34 Cho HH, Shin KK, Kim YJ et al. NF-kappaB activation stimulates osteogenic differentiation of mesenchymal stem cells derived from human adipose tissue by increasing TAZ expression. J Cell Physiol 2010;223:168–177.

35 Cervelli V, Gentile P, Scioli MG et al. Application of platelet-rich plasma to fat grafting during plastic surgical procedures: Clinical and in vitro evaluation. Tissue Eng Part C 2009;15: 625–634.

36 Anitua E, Sánchez M, Nurden AT et al. Autologous fibrin matrices: A potential source of biological mediators that modulate tendon cell activities. J Biomed Mater Res A 2006;77: 285–293.

37 Kevy SV, Jacobson MS. Comparison of methods for point of care preparation of autologous platelet gel. J Extra Corpor Technol 2004;36:28–35.

38 Siebrecht MA, De Rooij PP, Arm DM et al. Platelet concentrate increases bone ingrowth into porous hydroxyapatite. Orthopedics 2002; 25:169–172.

39 Waters JH., Roberts KC. Database review of possible factors influencing point-of-care platelet gel manufacture. J Extra Corpor Technol 2004;36:250.

40 Man D, Plosker H, Winland-Brown JE. The use of autologous platelet-rich plasma (platelet gel) and autologous platelet-poor plasma (fibrin glue) in cosmetic surgery. Plast Reconstr Surg 2001;107:229.

41 Marlovits S, Mousavi M, Gabler C et al. A new simplified technique for producing platelet-rich plasma: A short technical note. Eur Spine J 2004;13(suppl 1):S102–S106.

42 Lozada JL, Caplanis N, Proussaefs P et al. Platelet-rich plasma application in sinus graft surgery: Part I. Background and processing techniques. J Oral Implantol 2001;27:38.

43 Hood AG, Arm DM. Topical application of autogenous tissue growth factors for augmentation of structural bone graft fusion. Paper presented at: American Society of Extra-Corporeal Technology 11th Annual Symposium on New Advances in Blood Management, April 20–23, 2004; Las Vegas, NV.

44 Mazzucco L, Balbo V, Cattana E et al. Not every PRP-gel is born equal. Evaluation of growth factor availability for tissues through four PRP-gel preparations: Fibrinet, RegenPRP-Kit, Plateltex and one manual procedure. Vox Sang 2009;97:110–118.

45 Cervelli V, Gentile P, Grimaldi M. Regenerative surgery: Use of fat grafting combined with platelet-rich plasma for chronic lower-extremity ulcers. Aesthetic Plast Surg 2009;33: 340–345.

46 Cervelli V, Gentile P, Casciani CU. Use of platelet gel in chronic lower extremity ulcers. Plast Reconstr Surg 2009;123:122e–123e.

47 Cervelli V, De Angelis B, Lucarini L et al. Tissue regeneration in loss of substance on the lower limbs through use of platelet-rich plasma, stem cells from adipose tissue, and hyaluronic acid. Adv Skin Wound Care 2010;23: 262–272.

48 Crovetti G, Martinelli G, Issi M et al. Platelet gel for healing cutaneous chronic wounds. Transfus Apher Sci 2004;30:145–151.

49 McAleer JP, Kaplan E, Persich G. Efficacy of concentrated autologous platelet-derived growth factors in chronic lower extremity wounds. J Am Podiatr Med Assoc 2006;96: 482–488.

50 Rozman P, Bolta Z. Use of platelet growth factors in treating wounds and soft tissue injuries (review). Acta Dermatovenerol Alp Panonica Adriat 2007;16:156–165.

51 Kazakos K, Lyras DN, Verettas D et al. The use of autologous PRP gel as an aid in the management of acute trauma wounds. Injury 2009; 40:801–805.

52 Powell DM, Chang E, Farrior EH. Recovery from deep-plane rhytidectomy following unilateral wound treatment with autologous platelet gel: A pilot study. Arch Facial Plast Surg 2001;3:245–250.

53 Man D, Plosker H, Winland-Brown JE. The use of autologous platelet rich plasma (platelet gel) and autologous platelet poor plasma (fibrin glue) in cosmetic surgery. Plast Reconstr Surg 2001;107:229–237.

54 Guerrerosantos J. Evolution of technique: Face and neck lifting and fat injections. Clin Plast Surg 2008;35:663–676.

55 Guerrerosantos J, Guerrerosantos F, Orozco J. Classification and treatment of facial tissue atrophy in Parry-Romberg disease. Aesthetic Plast Surg 2007;31:424–434.

56 Cervelli V, Gentile P. Use of cell fat mixed with platelet gel in progressive hemifacial atrophy. Aesthetic Plast Surg 2009;33:22–27.

57 Grimaldi M, Gentile P, Labardi L et al. Lipostructure technique in Romberg syndrome. J Craniofac Surg 2008;19:1089–1091.

58 Coleman SR. Long-term survival of fat transplants: Controlled demonstrations. Aesthetic Plast Surg 1995;19:421–425.

59 Azzena B, Mazzoleni F, Abatangelo G et al. Autologous platelet-rich plasma as an adipocyte in vivo delivery system: Case report. Aesthetic Plast Surg 2008;32:155–158; discussion 159–161.

60 Matsumoto D, Sato K, Gonda K et al. Cell-assisted lipotransfer: Supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection. Tissue Eng 2006;12: 3375–3382.

61 Alvarez PD, García-Arranz M, Georgiev-Hristov T et al. A new bronchoscopic treatment of tracheomediastinal fistula using autologous adipose-derived stem cells. Thorax 2008;63: 374–376.

62 Yañez R, Lamana ML, García-Castro J et al. Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease. Stem Cells 2006;24:2582–2591.

63 Fang B, Song Y, Liao L et al. Favorable response to human adipose tissue-derived mesenchymal stem cells in steroid-refractory acute graft-versus-host disease. Transplant Proc 2007;39:3358–3362.