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TISSUE-SPECIFIC PROGENITOR AND STEM CELLS

Concise Review: Fat and Furious: Harnessing the Full Potential of Adipose-Derived Stromal Vascular Fraction

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ABSTRACT

Due to their capacity to self-renew, proliferate and generate multi-lineage cells, adult-derived stem cells offer great potential for use in regenerative therapies to stop and/or reverse degenerative diseases such as diabetes, heart failure, Alzheimer's disease and others. However, these subsets of cells can be isolated from different niches, each with differing potential for therapeutic applications. The stromal vascular fraction (SVF), a stem cell enriched and adipose-derived cell population, has garnered interest as a therapeutic in regenerative medicine due to its ability to secrete paracrine factors that accelerate endogenous repair, ease of accessibility and lack of identified major adverse effects. Thus, one can easily understand the rush to employ adipose-derived SVF to treat human disease. Perhaps faster than any other cell preparation, SVF is making its way to clinics worldwide, while critical preclinical research needed to establish SVF safety, efficacy and optimal, standardized clinical procedures are underway. Here, we will provide an overview of the current knowledge driving this phenomenon, its regulatory issues and existing studies, and propose potential unmapped applications. STEM CELLS TRANSLATIONAL MEDICINE *2017;6:1096–1108*

SIGNIFICANCE STATEMENT

Stromal vascular fraction (SVF) isolated from the adipose tissue has been used worldwide while research studies are underway. This scenario often generates conflicting rationales for treatments, confusing terms and general assumptions. Our contribution here is expected to advance the knowledge of scientists and clinicians about, specifically with respect to its composition, nomenclature and necessary studies. By doing so, we expect not only to clarify and extend the therapeutic potential of the SVF, but also present best practices and standards from other basic research fields that together will help accelerate the translation of future SVF research into patient care.

INTRODUCTION

More often than not, science reminds us that the discovery of therapeutic agents and drugs does not always follow a conventional, hypothesis driven path. In 1928, a future Nobel Prize laureate named Alexander Fleming returned from a two-week vacation to find mold on an accidentally contaminated, neglected *Staphylococcus* culture plate. Upon examination of the mold, he noticed that it unexpectedly prevented the growth of *Staphylococci*. Penicillin, the first naturally occurring antibiotic drug used therapeutically, had just been accidentally discovered [1]. More recently, a cGMP-specific phosphodiesterase type 5 inhibitor developed for the treatment of hypertension exhibited minimal therapeutic effect remedying angina pectoris (its original purpose). However, patients treated with this compound were returning for additional doses. This sought-after compound was Sildenafil, now known as Viagra, the first oral treatment approved to treat erectile dysfunction in the U.S. [2]. Similarly, recent evidence involving the therapeutic properties of stem cells, in particular those derived from adult tissues like bone marrow and adipose, may well place such cells in this selective group of discoveries that achieved unintended success outside their original purpose. Once prized for their differentiation capacity, adult-derived stem cells have consistently shown therapeutic properties that surpass their original realm of engraftment and replacement paradigms [3–5].

In this review, we will focus on stromal vascular fraction (SVF), a collection of nonexpanded,

heterogeneous cells derived from enzymatically digested adipose tissue and sometimes referred to as adipose-derived stem cells. Though not fully defined, SVF preparations are thought to encompass unknown numbers of stem cells; hematopoietic, adipose and endothelial progenitors; as well as immune cells, fibroblasts, pericytes, endothelial cells and other uncharacterized cells [6, 7]. In particular, SVF enriches for a particular population of stem cells, a subtype of mesenchymal stem cells (MSCs), which has gained much attention over the past decade for their therapeutic properties [4]. Due to its easy isolation, lack of ethical concerns and therapeutic potential, SVF has been rapidly gaining global attention. Basic research and clinical studies establishing safety, cell composition and efficacy are currently being undertaken. Such studies will help eradicate conflicting rationales for treatments, confusing terms and general assumptions. In this review, we will discuss nomenclature and regulatory issues, current applications and mechanisms of action, critical gaps in knowledge and potential unexploited clinical applications related to the use of SVF.

The Original Adipose-Derived Stem Cells

The isolation of stem cells from adipose tissue was originally described by Zuk and colleagues, who successfully isolated and cultured cells from human liposuction aspirates, ultimately naming them processed lipoaspirate or PLA cells. These adipose-derived cells shared the same characteristics of MSCs previously isolated from the bone marrow, exhibiting plastic adherence, fibroblast-like morphology, self-renewal, and capacity for multipotential differentiation [8–10]. Zuk suggested human PLA cells were perhaps a clonal variant of the MSC population located within the adipose compartment. Therefore, these multipotent adipose-derived cells could be used as an alternate therapeutic cell to MSCs, which, at that time, had been almost exclusively isolated from bone marrow aspirates [11]. Today we know that MSCs can be isolated from virtually any adult tissue with a stromal component [12], including umbilical cord and umbilical blood, placenta, fetal liver, muscle, lung, and gingival tissue [4, 13, 14]. In these niches, MSCs are thought to serve as progenitors for the skeletal tissue (bone, cartilage, and fat) [8], perivascular cells (although it has been shown that not all MSC can exert this function) [15, 16] and connective tissue cells [17]. But, its greatest impact has been witnessed outside the stromal niche, where expanded, infused MSCs have been consistently improving diseases in preclinical models of myocardial infarction, diabetes, wound healing, traumatic brain injury, sepsis, cancer, and other diseases through mechanisms not fully understood [18–30].

Not surprisingly due to its abundant availability, cells derived from adipose tissues are being heavily considered and used as a source of MSCs. According to the American Society for Aesthetic Plastic Surgery, close to 400,000 liposuction surgeries are performed per year, each one yielding 100 ml to $>$3 liters of lipoaspirate tissue [31]. More importantly, one can conveniently enrich for MSCs contained within the adipose tissue by virtue of enzymatic digestion, centrifugation and plastic adherence [32]. Following its first description in the literature, adipose-derived MSCs have been isolated by groups worldwide and have been titled adipose-derived stem/stromal cells (ASCs), adipose-derived adult stem cells, adipose-derived adult stromal cells, adipose-derived stromal cells, ASCs or adipose mesenchymal stem cells, lending confusion to the field [32]. In an effort to solve this issue, the International Federation for Adipose Therapeutics and Science (IFATS) reached a consensus and adopted the term "adipose-derived stem cells" (ASCs) to identify the isolated, plastic-adherent, multipotent, MSC-like cell population [32]. Therefore, following INFATS consensus, we will use the term ASCs when referring to MSCs isolated from the adipose tissue. Moreover, in a joint statement with the International Society for Cellular Therapy (ISCT), IFATS has also established minimal criteria and guidelines for the identification of SVF-derived ASC. Within the SVF, ASCs can be phenotypically identified as CD45$-$CD235a$-$CD31$-$CD34$+$. Cultured ASC can be identified, similarly to MSCs [33], as CD13$+$CD73$+$CD90$+$CD105$+$CD31$-$CD45$-$CD235a$-$, plastic adherent cells with tri-lineage differentiation potential. Phenotypically, however, ASCs differ from bone-marrow-derived MSCs in their positivity for CD36 and negativity for CD106 [6]. Interestingly, CD34 expression is found in the majority of SVF cells (up to 80%) [34], and two days after initial SVF plating, more than 95% of adherent ASCs express CD34 [35]. But, like observed in MSCs [36], its expression in ASCs is thought to be lost during in vitro expansion [37], indicating culture conditions affects affect the physiological phenotype of MSCs and ASCs. Differences between CD34$+$ and CD34$-$ populations and the importance of CD34 expression for the functionality of SVF and ASCs are extensively discussed elsewhere [34, 37, 38].

The SVF: Rise and Risk

The standardization and utilization of SVF in research and clinical settings has been problematic. Numerous available systems using either enzymatic or nonenzymatic adipose tissue-derived cell isolation have become commercially available (reviewed here [39]). Yet, despite its proven heterogeneous cell composition and lack of pre-clinical studies addressing safety and efficacy, enriched SVF has been collectively named stem cells and indiscriminately used in so called "stem cell clinics" around the U.S. The principal rationale behind the clinical use of SVF preparations relies on the existence of MSCs. However, it is known that SVF contains a diminutive percentage of MSCs, estimated at 2%–10% of the SVF [40]. Thus, clinical trials employing MSCs, regardless of tissue source, require their isolation and further in vitro expansion, as opposed to the use of freshly isolated SVF used in many clinics. In addition, the non-MSC component of the SVF contains populations of adipose, endothelial and hematopoietic stem/progenitor cells that have yet to be characterized. These cell types are functionally distinct from MSCs, but may share common markers such as CD34, which could encompass 63% of SVF cells, the majority of which are not MSCs [34]. These factors advance misperceptions surrounding composition of the SVF and further confound the field and the general public.

Although the use of autologous cells at the point-of-care (treatment occurs in the same surgical procedure) minimizes potential risks associated with SVF transplants, a series of intrinsic and extrinsic risk factors still ought to be considered. The presence of a heterogeneous population of cells with intrinsic progenitor potential immediately raises concerns about neoplasm and unwanted tissue differentiation. Tissue mass growth following transplantation of autologous stem cell from blood [41, 42] and olfactory mucosa [43] has been reported in patients. A recent systematic review of the available literature indicated the existence of a significant cancer-promoting property of ASC [44], although

no distinction between ASCs and SVF was made. Specifically for SVF, it has been shown that coinjection of noncultured, human CD34+ cells purified from lipotransfer procedures and breast cancer cells increased tumor growth in immunodeficient mice, and such growth was not due to the generation of adipocytes [45]. A recent study has shown that SVF injected intradiscally in a goat model of intervertebral disc regeneration caused severe inflammation characterized by lymphocytic infiltration, neovascularization, and endplate destruction [46], although the authors could not define the underlying destructive mechanism.

Extrinsic risk factors to autologous, nonmanipulated SVF preparations can potentially arise from cell handling (compromised sterility and variable cell yield), as well as dose and mode of administration. Sample contamination and cell yield may heavily depend on the isolation method used (e.g., closed versus open system, mechanic versus enzymatic isolation—reviewed here [47]). Unfortunately, there is insufficient data to establish a reliable dose versus effect relationship. Therefore, high dose of cells may be needed for therapy, increasing the risk of unwanted effects. Finally, concentrated cells may form aggregates and lead to pulmonary emboli or infarctions following administration [48], especially when intravenously infused, as demonstrated in hematopoietic stem cell (HSC) transplants [49]. A recent study showed no serious side effects, systemic infection or cancer was associated with SVF cell therapy in a total of 1114 patients who received SVF for the treatment of osteoarthritis [50], indicating a safe profile of SVF, at least following intra-articular injections for the treatment of joints. Additional studies will further address SVF risks and address important questions like postdelivery survival of cells, rejection and functional properties of the cells.

Regulatory Issues

In the U.S., the unregulated use of SVF in private clinics led to the intervention of the U.S. governing body responsible for supervising and regulating cell therapy, the Food and Drug Administration (FDA). The FDA currently defines SVF as a drug, device, and/or biologic product based on multiple criteria. First, the use of adipose-derived tissue for nonadipose related conditions is considered nonhomologous, meaning the "stem cells" do not perform the basic function or functions in the recipient as in the donor [51]. According to Code of Federal Regulations (CFR) Title 21 established by FDA, adipose tissue is classified as structural tissue that is intended to cushion and support other tissues. When the SVF is reintroduced into the body for purposes other than this intended use, this nonhomologous use must follow a 351 drug regulatory pathway. Second, the manufacturing steps required to produce SVF involves the use of collagenase enzymatic digestion, which classifies SVF as more than "minimally manipulated cells" according to the 21 CFR 1271.10(a) (1) criterion of minimal manipulation. During the digestion process, the structural components from the adipose tissue are removed, resulting in a manipulated product. There again, use of SVF falls outside of its natural biologic function and should comply with the FDA 351 drug regulatory pathway.

Exemption from the drug pathway occurs when the harvesting of the adipose tissue is processed during the "same surgical procedure." Point-of-care centrifuge machines that process adipose tissue for the production of SVF intended to be used for specific clinical applications must follow a 361 device regulatory pathway, which is regulated by guidelines posted in the 21 CFR part 1271. Depending on the initial regulatory pathway, a biologics license (drug) from the FDA or premarket approval (device) could be required prior to commercialization.

In Japan, "The Act on the Safety of Regenerative Medicine" which regulates medical professionals' practices and clinical studies related to regenerative medicine classifies SVF as a low risk or medium risk depending upon the level of risk associated with the medical treatment. Regardless of risk category, the regenerative medicine plan which should include using SVF must be submitted to the Ministry of Health, Labour and Welfare (MHLW). Additionally, any healthcare organization that wishes to offer regenerative medicine treatments must request opinions from a certified special committee or a certified committee on regenerative medicine about their plan before they can submit the plan to the MHLW. The most important step after the MHLW approval is to self-report adverse events and other specific plan details to the committees and MHLW [52].

In Europe, MSCs are classified as advanced therapy medicine products (ATMPs) guided through the European Medicines Agency (EMA). According to Directive 2004/23/EC and 1394/2007, in procedures in which SVF is autologous, cell administration is conducted in the same surgical procedure, and the essential function of cells is considered to be the same as in the donor's fat tissue, the cellular therapy treatment is not considered an ATMP. However, SVF can be classified as ATMPs by the EMA in nonhomologous applications, such as repair of injured tissues in case of nonhealing wounds and scarred tissue or in cases in which the SVF is combined with other products or cell types like MSCs. Additionally, the final product does not need to be placed on the market in the Member States as long as the cells are not being commercialized or sold to outside parties (http://www.ema.europa.eu/ema).

In Australia, legislative framework for the regulation of human cell and tissue products by the "Therapeutic Good Administration" (TGA) allows products that are derived from human tissue and cells during medical procedures that are considered a part of medical practice to be excluded from regulation provided they are collected from a patient who is under the clinical care and treatment of a licensed medical provider and manufactured by that medical provider for the therapeutic application in the treatment of a single indication and in a single course of treatment of that patient by the same medical provider, or by a person or persons under the professional supervision of the same medical provider. Thus, SVF is exempt from regulation in Australia based on the criteria discussed previously (https://www.tga.gov.au).

In comparison, the U.S. holds the most stringent regulations for SVF. Regardless of the regulatory direction in which SVF can be clinically used (351 drug regulatory pathway or the 361 device regulatory pathway), the final SVF product combines different cell types, which raises new clinical safety concerns as discussed above. Therefore, before SVF is clinically or commercially available, a series of stringent preclinical studies needs to be addressed, including cell characterization, manufacturing validation, safety studies and proof of pharmacological activity. These and other considerations have been comprehensively reviewed elsewhere [53]. Lack of such studies raises serious ethical considerations on the use of stem cell-based therapies as emphasized by Niemansburg [54] and Vonk and respectively colleagues [55]. A risk-benefit ratio becomes difficult to predict since the final SVF product is not well described, negative results are often unpublished and there is no defined mechanism of action to define endpoint

Table 1. List of clinical trials utilizing SVF currently listed at http://clinicaltrials.gov

Disease/ condition	Study status (Number of enrolled patients)	Therapeutic injection composition/ combination	Outcome/adverse effects	Reference[a] (Country andregulation)
Alopecia	Recruiting (8)	SVF injection into a 2 × 2 cm area	Ongoing	NCT02626780 (United States: FDA)
Osteoarthritis	Complete (6) phase 1	SVF injected directly into the intra-articular space with a mean of 12×10^6 viable nucleated SVF cells per knee	Improvement in WOMAC and VAS scores. No adverse effects.	NCT02357485 (United States: IRB)
	Complete (6) phase 1	SVF with a mean of 48 million nucleated SVF cells and a mean viability of 78%	Decreased pain and increased motility 12 wk post-op. No adverse effects	NCT02276833 (United States: IRB)
	Recruiting (100) phase 1/2	Intra-articular administration of SVF and PRP	Ongoing	NCT01739504 (United States: IRB)
	Complete (30) phase 1/2	10–50 10^6 SVF cells and 5–10 ml PRP	No results posted	NCT02142842 (Vietnam)
	Recruiting (39)	High and low Dose with Placebo control	Ongoing	NCT02726945 (United States: FDA)
	Recruiting (30)	Direct injection of SVF and activated platelets	Ongoing	NCT01947348 (United States: IRB)
	Recruiting (20)	Intra-articular injection of SVF	Ongoing	NCT02697682 (Denmark)
Breast cancer-related lymphedema	Recruiting (10) phase 2	Freshly isolated SVF in a cell-assisted lipotransfer to the affected axillary region	Ongoing	NCT02592213 (Denmark)
Pressure ulcers	Recruiting (12) phase 1	5.0×10^6 ASCs per cm^3 of wound area	Ongoing	NCT02375802 (United States: FDA)
Crohn's disease	Recruiting (10) phase 1/2	SVF microinjected around fistulas	Ongoing	NCT02520843 (France)
Erectile dysfunction	Recruiting (100) phase 1/2	Laboratory isolated SVF and PRP from peripheral blood	Ongoing	NCT02087397 (United States: IRB)
Diabetes mellitus type II	Recruiting (100) phase 1/2	Intravenous infusion of adipose derived SVF	Ongoing	NCT01453751 (United States: IRB)
Multiple sclerosis	Recruiting (50) phase 1/2	Intravenous infusion of adipose derived SVF	Ongoing	NCT01453764 (United States: IRB)
COPD	Recruiting (100)	Intravenous injection	Ongoing	NCT02041000 (United States: IRB)
	Recruiting (20) phase 1/2	Intravenous transfusion mixed with PRP	Ongoing	NCT02645305 (Vietnam)
	Recruiting (60) phase 1	Intravenous injection of SVF cells in saline solution	Ongoing	NCT02161744 (United States: IRB)
	Recruiting (100) phase 1/2	Intravenous infusion and inhalation delivery	Ongoing	NCT01559051 (United States: IRB)
Degenerative disc disease	Recruiting (100)	ASCs in combination with PRP	Ongoing	NCT02097862 (United States: IRB)
High tibial osteotomy	Recruiting (52)	3cc transplantation	Ongoing	NCT02642848 (Korea)
Micromastia	Recruiting (20) phase 2	SVF and autologous adipose	Ongoing	NCT02116933 (United States: IRB)
Adipose graft	Complete (20) phase 2	SVF and autologous adipose	No conclusions. No adverse effects	NCT01771913 (Brazil)
	Recruiting (30) phase 1/2	SVF and autologous adipose	Ongoing	NCT02076022 (United States: FDA)
Facial adipose graft	Recruiting (34) phase 2	SVF and autologous adipose	Ongoing	NCT02526576 (United States: FDA)
	Complete (6) phase 1	SVF and autologous adipose	No outcomes listed	NCT01828723 (United States: FDA and IRB)
Skin graft	Recruiting (75) phase 1/2	1×10^6 SVF transplanted per square centimeter	Ongoing	NCT02546882 (China)
Systemic sclerosis	Completed (12)	SVF injection into fingers	Improvement in hand disability No adverse effects.	NCT01813279 (France)
	Recruiting (40) phase 2	1 ml SVF	Ongoing	NCT02558543 (France)

(continued)

Table 1. *continued*

Disease/ condition	Study status (Number of enrolled patients)	Therapeutic injection composition/ combination	Outcome/adverse effects	Reference[a] (Country andregulation)
Critical limb ischemia	Recruiting (20)	SVF injected intramuscularly-30cc of SVF	Ongoing	NCT02234778 (United States: FDA)
Peripheral artery disease	Recruiting (10) phase 1	200×10^6 cells split into thirds. 1/3 intravenous injection, 1/3 intra-adventitia, 1/3 intra-muscular	Ongoing	NCT02756884 (United States: FDA)
Refractory rheumatoid arthritis, systemic lupus erythematosus or Sharp's syndrome	Recruiting (20) phase 1	Intravenous injection of SVF	Ongoing	NCT02741362 (United States: IRB)
Soft tissue defects/Abnormal healing wounds	Recruiting (10) phase 1	Subcutaneous injection with or without unprocessed autologous adipose	Ongoing	NCT02590042 (Canada)

[a]clinicaltrials.gov ID number of actively recruiting or completed interventional studies involving stromal vascular fraction or adipose derived stromal cells clearly specified as being the same as the heterogeneous, nonexpanded fractioned SVF.
Abbreviations: ASC, adipose-derived stem/stromal cells; COPD, Chronic Obstructive Pulmonary Disease; FDA, Food and Drug Administration; IRB, Institutional Review Board; SVF, stromal vascular fraction; PRP, Platelet-Rich Plasma.

measurements apart from clinical outcome. And for many conditions, the existence of an alternative, approved treatment hinders the availability of control groups in double-blinded randomized controlled trial.

Current Clinical Studies

A search on http://clinicaltrials.gov was performed (Search terms: Stromal vascular fraction, Adipose derived stromal cells, Adipose derived regenerative cells, Adipose derived stromal vascular fraction and Adipose derived stem cells). Studies with unknown status and MSCs only were excluded (Table 1). Studies within the U/S. are regulated by either the Institutional Review Board (IRB) or the FDA. FDA-approved studies in the U.S. indicate the presence of an investigational new drug or an investigational device exemption. Regulatory agencies in Korea and China are also named IRB and FDA, respectively. Regulatory agencies in other countries include: The Scientific and Ethic Board (Vietnam), The Regional Scientific Ethical Committees for Southern Denmark (Denmark), Agence Nationale de Sécurité du Médicament et des produits de santé (France), the National Committee of Ethics in Research (Brazil) and Health Canada (Canada)."

Therapeutic Potential of SVF

In general, SVF is thought to regenerate tissue through a variety of mechanisms. SVF has been shown to promote angiogenesis, partially through secretion of various growth factors such as vascular endothelial growth factor (VEGF) [56], the presence of endothelial progenitor cells (EPCs) [57] and the supportive role of ASC with pericytic properties [35]. Interestingly, using SVF embedded in Matrigel, Koh and colleagues have shown that SVF promotes neovascularization not through angiogenesis, but instead through reassembly of its endothelial cells into pre-existing vasculature. Moreover, this effect was dependent on the presence of macrophages [58], suggesting the presence of different cell types in the SVF might be beneficial. SVF was also shown to display anti-inflammatory effects in models of ischemic heart failure [59] and experimental autoimmune encephalomyelitis [60], although the mechanism(s) through which SVF can inhibit inflammation, apart from having an ASC population, remains speculative. Curiously, Blaber and colleagues, through in vitro cytokine analysis, have shown that SVF preparations secreted higher levels of IL-1β, IL-8, and IL-15, and lower levels of the anti-inflammatory cytokines IL-10 and IL-13 when compared to ASCs, suggesting SVF may possess distinct immunomodulatory properties. Finally, in vivo differentiation of SVF has been limited to fat graft retention studies, in which some SVF cells differentiated into adipocytes [61]. Furthermore, due to its heterogeneous composition of cells and still unknown effects from cross-talk between the different cells in SVF and between SVF and host tissue, defining the therapeutic properties of the SVF will be challenging. However, one can attempt to define its real therapeutic potential by separately assessing the potential of each cell component that has been identified within the SVF to date.

Mesenchymal Stem Cells

As the most studied and characterized cell from the SVF, MSCs first appeared in the annals of science in the 1860s thanks to the German pathologist Julius Cohnheim. Cohnheim and colleagues demonstrated the existence of nonhematopoietic, plastic adherent, fibroblast-like cells from the bone marrow, proposing that these cells were involved in the wound healing process [62]. In 1869, Goujon observed that bone marrow from rabbits and chickens could create ossification sites when transplanted into muscle [63]. It was not until the 1960s and 1970s that scientists revisited the subject. First, Tavassoli and Crosby demonstrated that fragments of bone marrow deprived of bone contained cells with osteogenic potential [64], and Friedenstein and colleagues confirmed the existence of such cells in a minor subpopulation of the bone marrow and coined the term colony-forming unit fibroblastic or CFU-Fs, identifying the cells based on their ability to form colonies derived from single cells [65]. In 1991, the bone marrow-

Table 2. List of factors implicated in the therapeutic effects of mesenchymal stem cells in experimental models further confirmed by suppression of gene expression or neutralizing antibodies

Factor	Experimental model
Prostaglandin E2 (PGE2)	Experimental arthritis [76],[77], atopic dermatitis [78], myocardial infarct [79], sepsis [80]
Tumor necrosis factor-inducible gene 6 protein (TSG-6)	Myocardial infarct [27], diabetes [24], corneal injury [81], peritonitis [19],[82] acute lung injury [20]
Indoleamine 2,3-dioxygenase (IDO)	Renal allograft model [83]
Hepatic growth factor (HGF)	Acute lung injury [84], Multiple sclerosis [85]
Vascular endothelial growth factor (VEGF)	Hyperoxic lung injury [86], Acute kidney injury [87]
Insulin growth factor (IGF)	Cisplatin-induced kidney injury [88]
Antimicrobial peptide LL-37	Pneumonia [25]
Transforming growth factor beta (TGF-beta)	Atopic dermatitis [78], Brain ischemia [89]

derived MSC (BM-MSC) differentiation paradigm was further expanded when Caplan and colleagues were able to differentiate these cells into osteoblasts, chondrocytes and adipocytes [8]. Based on this rather limited differentiation potential, CFU-Fs were named MSCs, still the most prevalent name when referring to these cells and the rationale behind the first studies involving MSCs.

Early studies involving BM-MSCs clearly indicated disease amelioration in models of bone repair, spinal cord injury, myocardial infarct, and diabetes. Despite significant failure to engraft and differentiate, these studies suggested BM-MSCs could improve disease outcome outside the therapeutic paradigm of tissue replacement [21, 22, 66–68]. The existence of another therapeutic paradigm in the BM-MSC field was confirmed by LeBlanc and colleagues, who cotransplanted in vitro-expanded BM-MSCs during a bone marrow transplant, based on the work of Frassoni et al. [69] and her own preclinical studies demonstrating that BM-MSC could inhibit lymphocyte activation in culture [70]. The addition of BM-MSCs significantly inhibited the development of graft versus host disease following transplantation [26], consolidating the concept of immunomodulation by BM-MSCs. Further studies have revealed that BM-MSC infusions, even allogeneic in nature, do not elicit immune responses in part because of a lack of immune costimulatory molecules and low expression of MHC class II [71]. Once infused, BM-MSCs have been shown to interact and modulate immune cells, skewing their activation and phenotype away from an inflammatory response (recently reviewed elsewhere [72]).

As aforementioned, MSCs can be isolated from virtually any adult tissue, based on the criteria established by the ISCT. It is now appreciated that the plasticity associated with MSCs lies within their capacity to sense and discretely respond to the environment, most likely exerting their therapeutic effects through secretion of paracrine cytokines and growth factors that modulate immune responses and endogenous repair (Table 2). More recently, BM-MSC-derived microvesicles have also been proposed to have therapeutic benefit [73–75]. Such treatment potential explains why MSCs are the subject of over 600 clinical trials at the time of this review according to http://clinicaltrials.gov, targeting a wide array of diseases.

It has not yet been fully elucidated whether or not ASCs and MSCs from different sources share the same therapeutic potential. Comparison between MSCs and ASCs remains a topic of intense debate [90]. Studies have shown that, apart from selected surface markers and trilineage differentiation, ASCs, like their counterpart MSCs, share a strong immunosuppressive capability [91], partially through secretion of exosomes [92], anti-apoptotic [93] and anti-scarring effects [94], as well as their ability to secrete trophic factors like VEGF, HGF and TGF-beta [56]. However, transcriptome and proteome analysis revealed 13.2% and 18% targets, respectively, were differentially expressed between MSCs and ASCs [90], indicating intrinsic differences between these cell types. Nevertheless, the use of ASCs, like MSCs, has been proposed for the treatment of a multitude of conditions such as cardiovascular diseases [95], autoimmune disorders [96], and tissue engineering [97]. A few studies have compared the therapeutic properties of MSCs isolated from different tissues. Noel and colleagues' work supports the use of MSCs over ASCs for osteogenesis and chondrogenesis based on a pre-commitment of MSCs toward such lineages [98]. Heo and colleagues did not observe any significant differences in growth rate, colony-forming efficiency and immunophenotype from ASCs or MSCs derived from bone marrow, placenta, and umbilical cord blood [99]. Interestingly, only bone marrow and ASCs significantly inhibited mitogenic T cell proliferation [99]. In this regard, Keyser and colleagues have shown that murine ASCs and MSCs isolated from muscle tissue, omentum, and bone could inhibit mitogenic and allogeneic T cell activation regardless of tissue of origin. However, this inhibition was most pronounced for muscle-derived MSCs and ASCs in the mitogen and allogeneic T cell activation respectively [100]. Last, a recent study comparing MSCs from bone marrow and ASCs proposed that the latter had more potent immunomodulatory potential since ASCs displayed increased Indoleamine 2, 3-dioxygenase activity and Prostaglandin E2 expression [101]. These differences can be attributed to the existence of inconsistent protocols for cell isolation, expansion and freezing. It is known that culture conditions, such as fetal bovine serum, human supplements, cell seeding density and oxygen conditions, can influence the quality, proliferation, senescence, and immunomodulation ability of the cells (reviewed elsewhere [102]). Further research is needed for the establishment of rigorous potency assays, quality control and culture standards.

Endothelial Progenitor Cells

EPCs are required for vasculogenesis during early embryo development. In contrast, adult vascular growth develops from fully-differentiated endothelial cells through angiogenesis [103]. However, additional findings have shown the existence of postnatal, circulating EPCs that share phenotypic characteristics with their embryonic counterpart, proposing an angiogenic role for EPCs [104, 105]. EPC mobilization and possible engraftment have indeed been confirmed in postnatal angiogenesis in the presence

of coronary artery disease and myocardial infarction [106]. It is also known that their circulating and wound level numbers are decreased in diabetes [107]. Hence, numerous clinical trials have been conducted in patients with heart disease, diabetes, peripheral arterial disease, pulmonary disease, and cancer in which putative EPCs have been examined as a biomarker or used as cell therapy (http://clinicaltrials.gov). Unfortunately, angiogenic therapies using EPCs have been largely unsuccessful to date. Endogenous EPC recruitment elicited by angiogenic factors like VEGF is insufficient to cause effectual angiogenesis and the use of allogeneic EPCs leads to immune rejection and poor transplantation outcome [108, 109]. Thus, the use of autologous SVF, with EPCs and endothelial cells at numbers varying from 7% to 30% [40, 110], provides a rationale for the use of SVF in the treatment of diseases with a pathogenic vascular component. Due to the potentially large numbers of EPCs that can be isolated from the SVF, it is possible that the SVF may constitute a superior source when compared to whole blood. But before SVF-derived EPCs are considered for angiogenic therapies, some challenges need to be overcome. First, there are no definitive markers for identification of EPCs. Current EPC marker combinations include $CD31^+/CD34^+/CD90^+$ [110], $CD31^+CD34^+CD45^-CD90^+CD105^{low}CD146^+$ [40] (adipose tissue) and $CD34^+KDR^+CD133^+$ [111] (circulating EPCs). Using rat adipose tissue, Zhou and colleagues isolated and cultured EPCs that were characterized as CD34+Stro-1+ VEGFR-2+ eNOS+CD31+α-SMA-CD14−CD45−. These EPCs formed capillary-like structures in static Matrigel and acellular biological scaffold and secreted VEGF, supporting an angiogenic role for EPCs [112]. Hager and colleagues combined CD31, CD144, VEGF-R2, CD146, CD73, and CD105 to isolate rare human EPCs that could differentiate into endothelial cells in vitro [113]. Perhaps more importantly, it is known that in vitro cultures of blood yield two distinct subpopulations of EPCs: early EPCs (eEPCs) and outgrowth endothelial cells (OECs) [114]. In a comparative study using transcriptomic, proteomic and structural analysis, eEPCs were shown to closely resemble monocytes, while OEC expression correlated with endothelial cells [115]. This finding is significant because eEPCs were shown to express genes involved in inflammation and immune responses [115], potentially eliciting or exacerbating strong inflammatory responses. Moreover, these findings might explain the high rates of immune rejection seen with allogeneic EPC transplants [108, 109]. Interestingly, culture dishes containing the same media can be used to isolate these cells by modifying its coating agent; fibronectin will select for eEPCs, while collagen will isolate OECs [116]. Therefore, defining the composition and phenotype of the EPCs in the SVF is necessary to reveal its full potential.

Hematopoietic Stem Cells

By definition, a HSC is a cell isolated from the blood or bone marrow that can renew itself and differentiate to a variety of specialized cells within the hematopoietic lineage [117]. HSC transplantation has been used to treat a variety of blood-related conditions, including destruction of cancerous hematopoietic cells, inherited anemia, and, most recently, autoimmune diseases [117, 118]. Classically, the bone marrow, umbilical cord, or peripheral blood collection after stimulation with granulocyte colony stimulating factor (G-CSF) have been the tissue sources for HSC transplants. But, the presence of HSCs suggests the SVF could provide a reliable cell source in human leukocyte antigen (HLA)-matched HSC transplants. Once again, caution is required before adipose-derived HSCs are considered for clinical applications. Animal studies strongly suggest the existence of two distinct HSC populations within the hematopoietic niche: a long-term and a short-term HSC. Long-term HSCs are capable of self-renewal throughout the lifespan of an organism, while short term HSC, at least in rodents, may only restore hematopoiesis for a few months [119]. Unfortunately, there are no definitive assays capable of identifying long term HSCs. Advanced methods for isolating mouse long-term HSCs combine the use of Rhodamine-123 (Rho) and/or Hoechst 33342 efflux measurements, or antibody combinations against CD48, CD150 and $Lin^-Sca1^+Kit^+CD34^-Flt3^-$ (reviewed elsewhere [117]). Human long term HSCs have been isolated using Thy1+RholoCD49f+ [120]. Still, the gold standard in identifying the phenotype of HSCs is transplantation and reconstitution of the bone marrow HSC population in rodents following irradiation [121]. In this regard, Han and colleagues (2010) have demonstrated the existence of HSCs in the SVF of rodents in the frequency of 0.004% ± 0.001% using Lin-Sca-1^+c-kit^+ and confirmed the long-term multilineage reconstitution ability of the SVF after transplant [122]. Colony-forming cell assays using the SVF from recipient mice revealed that all SVF-HSCs originated from the bone marrow. Further, HSC mobilization using G-CSF increased the number of functional HSC in the SVF [122]. These results support the use of SVF as an alternative source of HSCs. It is likely that the longevity and phenotype of HSCs in human SVF could be elucidated using similar phenotypic profiles and/or animal models.

Intriguingly, there are reports of HSC plasticity toward nonhematopoietic cells. Using lethally irradiated female FAH(−/−) mouse, an animal model of tyrosinemia type I, Lagasse and colleagues showed that transplantation of as few as 50 male HSCs led to abundant growth of donor-derived liver cells in recipient female mice [123]. Recently, Krause and colleagues identified epithelial cells derived from donor HSCs in the lungs, gut and skin of recipient mice [124]. Human studies have demonstrated male liver cells in female patients who have received bone marrow grafts from male donors and vice versa, suggesting that some bone marrow-derived cells have the capacity to integrate into the liver and form hepatocytes [125]. While these findings are of interest, additional studies are required to define the differentiation capacity of HSCs under various physiological and pathological conditions.

Immune Cells

It is known that the SVF contains monocytes and macrophages. It is estimated that the monocyte/macrophage compartment constitutes approximately 10% of the SVF, based on CD14 expression [126]. Macrophages found in the SVF express phenotypical markers of M2 macrophages (CD163 and integrin αvβ5) and secrete IL-10 and IL-1 receptor antagonist [127]. This M2 phenotype opposes their immune counterpart M1 macrophages, which have been historically understood to mediate inflammatory responses [128]. M2 macrophages are thought to exert anti-inflammatory functions and therefore offer a novel therapeutic opportunity. Animal models indicate modulating macrophages toward an M2 phenotype can inhibit the recruitment of inflammatory cells and is associated with significant protection against atherosclerosis [129, 130]. A recent clinical trial showed that stroke patients who received autologous M2 macrophages significantly improved their neurological recovery, in part through the immunomodulatory activity of M2 macrophages [131]. However, the

phenotype of macrophages needs further characterization. Studies indicate monocytes/macrophages present in the adipose tissue are significantly affected by obesity. It is known that obesity induces an accumulation of macrophages in the adipose SVF. These accumulated macrophages appear to be of M1 phenotype and closely associate with chronic inflammation in part by producing pro-inflammatory molecules [132]. In fact, a recent study showed that adipose-derived macrophages isolated from obese patients had a skewed monocyte/macrophage phenotype ratio, with higher number of macrophages expressing M1 markers when compared to nonobese patients [133]. Interestingly, postbariatric surgery patients displayed reduced M1 accumulation when compared to presurgery levels, supporting the notion that the inflammatory environment is driven by adipose accumulation. Therefore, the macrophage composition of individual patient SVF and its influence on modulating inflammation must be taken into consideration [133].

Regulatory T-cells (Tregs) are an immunosuppressive subpopulation of T-cells that inhibit the induction and proliferation of effector T-cells, thereby modulating autoimmunity, allergic responses, inflammation and responses to infections and tumors [134]. Tregs comprise approximately 5%–20% of the $CD4^+$ T-cell compartment, but their numbers in the adipose tissue are still unknown. Studies in rodents indicate key differences between visceral adipose tissue-resident Treg cells, also known as "Fat Tregs" and lymphoid-derived Tregs. Fat Tregs account for a much larger fraction of $CD4^+$ T cells (50%–70%) [135]. In addition, Fat Tregs differentially express many genes in comparison to lymphoid-derived Tregs. While Fat Tregs maintain approximately 60% of the canonical Treg signature, they differentially express genes that are mainly associated with lymphocyte migration, extravasation and lipid metabolism [135]. Interestingly, Tregs in adipose tissue express a much higher level of IL-10 (136-fold augmentation of IL-10 transcripts) in comparison with lymph node Tregs [136], supporting a higher anti-inflammatory potential for adipose-derived Tregs. Notably, the negative effects of obesity are also observed in these cells. Fat Tregs are abundant in visceral adipose tissue of lean mice, but their number is greatly reduced in insulin-resistant animal models of obesity [136, 137]. Taken together, the utilization of anti-inflammatory, immunomodulatory cells from the adipose tissue, although promising requires additional considerations.

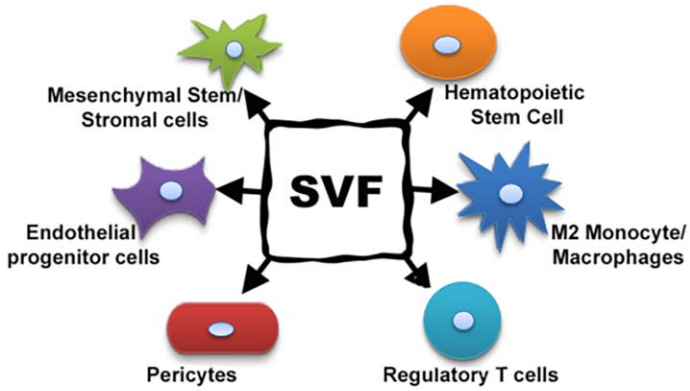
Pericytes

Blood vessels throughout the body are formed by two interacting cell types: endothelial cells and perivascular cells. Pericyte recruitment is essential for the maturation of the developing vasculature. Following primary capillary plexus formation, a functional vessel network is formed by extensive pruning and sprouting of vessels. Once newly formed sprouts cease proliferation, they secrete growth factors such as PDGF-B that attract pericytes to envelop vessels in the brain, kidney, heart, lung and adipose tissue [138]. Pericytes, like smooth muscle cells, function on large diameter vessels, and regulate blood flow by modulating vasoconstriction and vasodilation [139]. Interestingly, pericytes perform specific functions in different organs. The highest density of pericytes in the body is found in vessels of the central nervous system, where endothelial cells are covered with pericytes in a 1:1 to 3:1 ratio to form and protect the blood-brain barrier [139–141]. Although the density of pericytes in adipose tissue is not known, endothelial-to-pericyte ratios in normal tissues vary between 1:1 and 10:1. Pericyte coverage of the endothelial abluminal surface within adipose tissue ranges between 70% and 10% [142]. Interestingly, brain pericytes may constitute a microglia precursor with phagocytic activity [143]. In the liver, they are called hepatic stellate cells and are known to regulate extracellular matrix remodeling, vitamin A metabolism (containing more than 80% of the total vitamin A in the body) [144] and inflammatory cell recruitment resulting from liver diseases [145]. Kidney pericytes are critical for the increased capillary surface area caused by branching of a single invading vascular loop into the glomerular capillaries [146]. Due to tissue specificity and functional heterogeneity, there is no known single pericyte-specific marker. Proposed markers include a combination of $CD146^+CD34^-CD45^-CD56^-$, CD13, platelet derived growth factor receptor-β (PDGFR-β), epidermal growth factor receptor (EGFR), adenosine A2 receptors, α-smooth muscle actin, desmin, NG2 proteoglycan, aminopeptidase A, aminopeptidase N and regulator of G-protein signaling 5 (RGS5) [147].

Due to its vascular supporting role, pericytes have been considered for the treatment of diseases with a vascular component. A recent study showed transplantation of pericytes improved heart function, as measured by attenuated ventricular dilatation and improved contractility, reduced fibrosis, and diminished inflammation in an ischemic heart disease model [148]. However, very little donor engraftment was detected and therapeutic improvements were attributed to unknown paracrine factors and cellular interactions. Moreover, Katare and colleagues demonstrated saphenous vein-derived pericyte progenitor cells (SVPs) could improve heart function in a mouse myocardial infarction model through a concert of growth factors and chemokines [149]. Interestingly, SVPs transfected with siRNA against miR-132 exhibited reduced efficacy following myocardial infarct, indicating a critical therapeutic role for miR-132 [149]. Studies have shown that adipose-derived pericytes (CD146+, CD45−, CD34−, CD31−) have significant bone regeneration potential in an atrophic, nonunion model [150]. Another study used CD146+/CD34−/CD45−/CD56− pericytes and demonstrated increased lifespan in a mouse model of Duchenne muscular dystrophy [151]. Significantly, both studies showed no signs of differentiation. As one might expect, stress conditions significantly affect pericyte survival. Loss of pericytes is an early hallmark of diabetic retinopathy and leads to microaneurysm due to reduced vessel integrity [152]. Inducing inflammation with lipopolysaccharide treatment leads to pericyte loss and microvascular dysfunction in a mouse model of sepsis [153]. Furthermore, loss of pericytes accelerates Aβ accumulation, the appearance of Tau pathology and neuronal degeneration in mice overexpressing Aβ-precursor protein [154]. Given the critical role for pericytes in vascular structure and function, future studies could reveal a therapeutic role for pericytes in health and disease.

Conclusion

Given its abundance and mixture of potentially therapeutic cells, the treatment of various diseases and conditions with SVF-derived cell therapies holds great clinical promise. Along with the combined efforts from IFATS and ISCTs, scientist and clinicians should further emphasize the difference between cells from the SVF of the adipose tissue and the adipose tissue-derived stem cells, the ASCs. Such difference may not only dictate different therapeutic rationales given the ever-expanding therapeutic



Cell type	Frequency	Potential SVF marker	Potential application
Mesenchymal stem/stromal cells	2%-10%	CD45-CD235a-CD31-CD34+ [6]	Heart failure, diabetes, brain stroke, arthritis, dematitis, sepsis, multiple sclerosis, acute lung injury, allograft transplantation, kidney injury, peritonitis (Table 2)
Hematopoietic stem cells	~0.004%	Thy1+RholoCD49f+ [120]	Blood-related conditions, HSC reconstitution, inherited anemia, autoimmune diseases [117, 118]
M2 monocytes/ macrophages	~10%	CD14+CD163+ Integrinα5+ [127]	Atherosclerosis, stroke [129, 130, 131]
Regulatory T cells	5%-70% of CD4 T cells	CD4+CD25+Foxp3 [136]	Autoimmunity, allergy, inflammatory bowel disease, ischemia-reperfusion kidney injury, allograft rejection [135]
Pericytes	Unknown	CD146+, CD45−, CD34−, CD31 [150], CD146+/CD34-/CD45-/CD56- [151]	Ischemic heart disease , diabetic retinopathy and Duchenne muscular dystrophy [148, 150, 151]
Endothelial Progenitor cells	7%-30%	CD31+/CD34+/ CD90+ [110], D31+CD34+ CD45−CD90+CD105low CD146+ [40]	Heart disease, diabetes, peripheral arterial disease, pulmonary disease (clinicaltrials.gov)

Figure 1. Cellular subsets within the SVF. Abbreviations: HSC, hematopoietic stem cell; SVF, stromal vascular fraction.

properties of ASCs and the different cell components within the SVF, but also directly impacts therapy regulations, particularly in countries where the use of SVF could be placed under a less stringent regulation than ASCs or MSCs due to its autologous, point-of-care use. There is a great need to accelerate the knowledge of scientists and clinicians on SVF, specifically with respect to its composition, nomenclature and necessary studies. Many basic scientific questions remain to be addressed. Although the mechanisms through which SVF regenerates tissue remains inconclusive, the literature supports the contribution of paracrine effects, with crosstalk between SVF components and host leading to repair and healing. In this paradigm, differentiation may play a minor role. Future studies are needed to elucidate the mechanism(s) of action of SVF and their differentiation potential in vivo. The heterogeneity for different ASC and MSC preparations has been extensively discussed, including the work of Baer and colleagues highlighting ASC donor variability in forty-nine cellular surface markers in a comprehensive phenotyping study [155], and the work of Siegel and colleagues characterizing surface markers, proliferation capacity, and in vitro function from 53 different MSC preparations [156]. Further research needs to investigate donor variability in SVF preparations. Are there intrinsic differences in composition of SVF between donors? Studies that highlighted differences in specific subpopulations, like the inflammatory effects of obesity on macrophages [133] need to be expanded to other subpopulations in the SVF in order to understand how SVF composition differs in healthy versus disease states. These questions are particularly important to not only understand SVF biology, but to also reliably predict the therapeutic efficacy of SVF. Likewise, determining optimal dose/infusion

schedules and the development of potency assays will help optimize the therapeutic potential of SVF. Encouraging studies mentioned here describing the isolation and characterization of SVF-derived HSCs, EPCs and pericytes attest to significant therapeutic promise. Hence, are we underestimating the therapeutic potential of SVF? Can the SVF be used as an alternative source of endothelial precursors, HSCs, M2 macrophages, regulatory T cells and pericytes? Classically, these cells have been found in the bone marrow or blood in low frequencies, limiting their clinical utility. Using the SVF as an abundant source for such therapeutic cells could have profound effects for a myriad of conditions and diseases (Fig. 1). Finally, adverse effects from SVF treatments offered in private, unregulated clinics often go unreported or are sometimes only found in the news. Results from completed phase 1 clinical trials (Table 1) indicate SVF treatment for osteoarthritis, SLE and fat graft—in which SVF is locally injected; indicate a safe profile, with no short time neoplasma, unwanted tissue differentiation or adverse effects. Given the autologous, noncultured nature proposed for SVF therapies, tracking SVF cells following infusion poses a significant challenge. Using a protocol for labeling SVF cells with CS-1000, a perfluorocarbon and ^{19}F-rich agent, Rose and colleagues attempted to solve this problem by developing a labeling clinical protocol at the point-of-care [157]. However, the procedure required the use of red blood cell (RBC) lysis step and only 37% of the total SVF was labeled, with preferentially labeling of CD34+ cells over CD45+ cells. Current and future studies will elucidate the principal risks associated with SVF-based therapies, including where SVF-derived cells migrate and reside.

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Author Contributions

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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