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REVIEW ARTICLE

The Adipose Stromal Vascular Fraction as a Complex Cellular Source for Tissue Engineering Applications

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A major challenge in tissue engineering is the generation of sufficient volumes of viable tissue for organ transplant. The development of a stable, mature vasculature is required to sustain the metabolic and functional activities of engineered tissues. Adipose stromal vascular fraction (SVF) cells are an easily accessible, heterogeneous cell system comprised of endothelial cells, macrophages, pericytes, and various stem cell populations. Collectively, SVF has been shown to spontaneously form vessel-like networks *in vitro* and robust, patent, and functional vasculatures *in vivo*. Capitalizing on this ability, we and others have demonstrated adipose SVF's utility in generating and augmenting engineered liver, cardiac, and vascular tissues, to name a few. This review highlights the scientific origins of SVF, the use of SVF as a clinically relevant vascular source, various SVF constituents and their roles, and practical considerations associated with isolating SVF for various tissue engineering applications.

Keywords: stromal vascular fraction, vasculature, lipoaspirate, adipose tissue

Introduction

Efforts to engineer replacement tissues have yielded significant advances, from the production of simple conduits and reservoirs such as tracheas[1] and urethras[2] to the development of cardiac,[3] kidney,[4] and liver tissue mimics.[5–7] However, because complex organ systems require an operative vasculature to meet their metabolic demands, a key challenge facing tissue-engineered structures is the development and presence of a stable, functional vascular supply.

As the largest endocrine organ in the body,[8] adipose utilizes a complex vasculature to influence systemic processes, from insulin sensitivity to inflammation and immunological functions.[9,10] Autologous adipose tissue has been used to fill soft tissue defects for decades[11–14] and already has broad clinical relevance.[15–17] It can also be processed to yield a distinct, heterogeneous cellular mixture devoid of adipocytes known as the stromal vascular fraction (SVF),[18–21] which can self-assemble into complex vascular networks[22] that are hierarchical, highly branched, and perfused[6] (Fig. 1). This, in conjunction with the fact that SVF can be easily obtained from lipoaspirates at the point of care, makes it an attractive option for providing support to engineered tissues.[23]

Although several efforts have used adipose-derived homogeneous cell populations for stromal and/or vascular support, it is important to recognize that SVF is a complex cellular system—and not simply a uniform cell type—that has clinically relevant, therapeutic potential. The purpose of this work is not only to highlight the utilities of the various cell types within SVF, but also to distinguish it from other similar cell types and sources. From this, we will also discuss future avenues to consider to further enhance SVF's relevance in research and clinical endeavors.

A Concise History of SVF

In the mid 1960s, nutrition and endocrinology chemist Rodbell performed the first experiments that isolated SVF from rats.[18–21] Epididymal adipose tissue was exposed to collagenase before being centrifuged, resulting in three layers of cell populations with differing densities. The top layer was comprised of fat and buoyant adipocytes, whereas the middle layer contained tumescent fluid and excess collagenase solution. The cells at the very bottom—a pellet containing a mixture of cells (such as endothelial cells [EC], mast cells, stem cells, and macrophages)—formed the SVF.

While the subject of Rodbell's works during this time chiefly dealt with the metabolic properties of the adipocytes, his isolation of SVF led to subsequent pioneering efforts by Wagner *et al.* in the 1970s. In this setting, the adipose tissue was digested and centrifuged twice in an effort to isolate just the capillary EC, on which Wagner then performed studies to characterize the endothelial adenylate cyclase activity.[24] Later that decade, in 1975, Wagner and Mathews further perfected their isolation of capillary endothelium from adipose SVF by adding thimerosal to further eliminate nonEC

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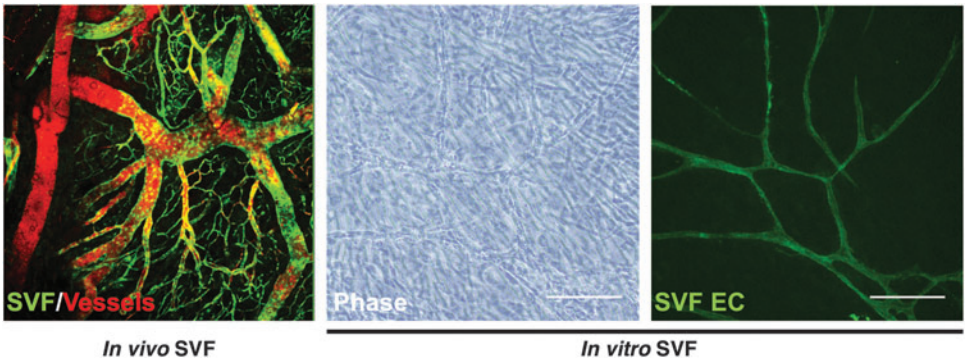

FIG. 1. *In vivo* and *In vitro* SVF microvasculature. SVF cells from GFP$^+$ rat were suspended in a three-dimensional collagen-I matrix and implanted subcutaneously for 4 weeks. SVF (*green* fluorescence) spontaneously self-assembled a hierarchical, mature, and patent vasculature. This vasculature anastomoses with the host circulation (GFP$^-$ Dextran-TRITC$^+$) and demonstrates evidence of perfusion (GFP$^+$ Dextran-TRITC$^+$). The ability of the SVF cells to self-organize into a functional vasculature has therapeutic implications in a multitude of tissue engineering applications (*Image credit: Dr. James Hoying*). When SVF cells are plated *in vitro*, the SVF-derived endothelial cells (*Ulex Europaeus Agglutinin-I*-fluorescein$^+$) spontaneously self-assemble an endothelial network within the overlying stromal cells. Scale bar = 10×, 200μm. SVF EC, stromal vascular fraction endothelial cells. Color images available online at www.liebertpub.com/teb

from the fraction.[25] These polygonal cells formed a monolayer in two-dimensional culture characterized by vesicles and numerous intracellular junctions.

Although Rodbell, and Wagner *et al.* used adipose tissue or adipose SVF derivatives to study the biochemical processes of individual cell types, their methods facilitated efforts that utilized SVF in its entirety. For instance, in 1976, Van *et al.* characterized the doubling time of cultured SVF cells as between 40 and 60 h.[26] In this setting, cultured SVF cells eventually formed a predominantly fibroblastic monolayer that also internalized lipids—a characteristic not observed with skin fibroblasts. Because these cells also synthesized and hydrolyzed triglycerides, a significant proportion of cultured SVF cells were thought to be adipocyte precursors.

In the late 1980s, Jarrell, Williams, and Rupnick, *et al.* isolated EC from human adipose SVF to endothelialize surgical vascular grafts.[27,28] This research was later expanded to include point-of-care applications in which SVF could theoretically be isolated and delivered at the bedside perioperatively.[29] Williams, Hoying, *et al.* also contributed to a greater understanding of the effects of enzymes on adipose digestion,[30] differences in site-specific adipose isolation[31] (also discussed in greater depth later-on in this review), and the formation of adipose-derived vascular networks in three-dimensional engineered structures[32]—all issues that critically affect the role and performance of SVF in tissue-engineered applications.

Thus, by the mid 1990s, SVF had been identified and several studies seeking to understand its capabilities and composition had already been conducted or were underway. The next section of this review will focus on several of the cell types present within SVF and their contributions to this dynamic mixture.

SVF Characterization and Isolation

SVF is a heterogeneous, versatile, and clinically relevant cell system. The interaction of these cell types contributes to SVF's overall therapeutic potential. Below, we discuss some of the cell types present as well as their discovered roles and mechanisms of action.

SVF composition

Today, SVF is known to have fibroblasts, mesenchymal stem cells (MSC), and EC, as well as smooth muscle cells, mural cells, macrophages, blood cells, and a whole cadre of other stem cell phenotypes (outlined in Table 1).[6,16,22,33–38] While this mixed population more closely recapitulates the variety of cells seen *in vivo*, there is an overall lack of consensus regarding the specific proportions of these constituents to one another.[39] Contributing to this is the fact that the SVF composition is dependent on a variety of factors, such as the adipose isolation site, processing methods, and the patient's own pathological status.[40]

In our initial analysis of the cell proportions within SVF, we processed rodent SVF cells through a fluorescence-activated cell sorting system to identify five populations: CD31$^+$ EC, CD14$^+$ monocytes and macrophages, CXCR4$^+$/cMet$^+$ multipotent cells, cKit$^+$ progenitor cells, and PDGFR-B$^+$ perivascular cells.[6] We took this a step further by then examining differences in the proportions of these cells in fresh versus cultured SVF isolates. We observed that ~33% of freshly isolated SVF cells were comprised of EC, and that culturing the SVF cells significantly reduced this number to ~10% of the total population. Similar trends were observed with CD14$^+$

Table 1. Identified Multicellular Composition of Adipose Stromal Vascular Fraction

Cell type	*References in this review*
Endothelial cells	6,22,31,35,36,41,43,45,48
Smooth muscle cells	6,22,31
Fibroblasts	6,22,31
Pericytes/perivascular cells/mural cells	6,22,31,37
Macrophages/monocytes/ lymphocytes/immune cells	6,22,31,35,41,75,76
Adipose (-derived) stem cells/ mesenchymal stem cells/ other stem cell phenotypes	6,31,33,35,36,49,50,67,68,69

monocytes/macrophages, which declined from ~22% to ~18%; with c-Kit$^+$ progenitors, from ~5% to ~1%; with CXCR4$^+$ multipotent cells, from ~2.5% to ~0%; and with PDGFR-B$^+$ perivascular cells, from ~20% to ~18%—all due to culturing. From this preliminary study and *in vivo* experiments utilizing both SVF populations, it was abundantly clear that culturing the cells for as little as one passage, profoundly altered their cellular composition and resulting vascular phenotype.

Morris *et al.* examined the SVF stromal cells with greater specificity, focusing particularly on the proportions of CD11b$^+$ innate immune cells, F4/80$^+$ tissue macrophages, Gr-1$^+$ myeloid cells, and CD2$^+$ lymphocytes in relation to the amount of Tie2-GFP$^+$ EC.[41] Of these, ~67% of the SVF cells were comprised of innate immune (~20%), endothelial (~25%), and myeloid cells (~22%). To compare, Dong *et al.* reported that SVF is rich in blood-derived cells, adipose stromal cells (CD34$^+$), and EC,[35] whereas Klar *et al.* divided SVF into four populations—EC (CD31, CD34, and CD146), MSC (CD44, CD73, CD90, and CD105), stem cells (CD49f, CD117, CD133), and myeloid hematopoietic stem cells (CD14, CD15, CD45).[36] To add to these descriptions, Silva *et al.* characterized the SVF adipose stromal/stem cell (ASC) population as being comprised of pericytes (CD45$^-$ CD146$^+$ CD34$^-$) and supra-adventitial preadipocyte-like cells (CD45$^-$ CD146$^-$ CD34$^+$).[37]

This constellation of markers and designations makes for a somewhat confusing mosaic, highlighting the need for a standardized marker set by which different groups can compare the efficiencies and compositions of their SVF isolations. Part of this perplexity can be attributed to a lack of specific, unique markers for cells such as ASC[39] or pericytes,[42] for example. Additionally, the use of cultured cells over fresh isolates inherently selects for cells that adhere to tissue culture plastic within a defined timeframe, potentially skewing the distribution of the various constituents. Despite these challenges, what is clear is that the SVF is a dynamic population of cells with a potentially significant clinical utility.

Endothelial cells

One of the hallmark characteristics of adipose SVF cells is their ability to self-assemble into a hierarchical, branched, perfused vasculature *in vivo*[6,22,43]—characteristics that are associated with vascular maturity.[44] While this capability requires a carefully orchestrated interplay between several cell types, the EC in SVF are critical to its formation of a functional vasculature.

In the early 1980s, Madri and Williams demonstrated that communications between the SVF EC and surrounding connective tissue dictated the cells' propensity to proliferate and form sheets, or to aggregate into tube-like structures.[45] The introduction of interstitial collagen to SVF EC cultures precipitated monolayer formation and, with sufficient time, the formation of tube-like structures. Yet, the presence of basement membrane collagen promoted SVF EC tube-like formations over their proliferation into monolayers. These findings suggested that cultured SVF EC phenotypes are regulated by connective tissue—a point corroborated by Montesano *et al.*, who used a clonal brain endothelial cell line instead of SVF EC.[46] These findings also indicated that SVF EC can assemble into networks *in vitro* in the absence of other cell types, although these capillary-like networks carry no blood, and that blood flow and pressure are not required for basic SVF EC network assembly and architecture.[6,47] Furthermore, with regard to *in vivo* assembly, Madri and Williams suggested that an angiogenic environment (such as a wound, for instance) may provide the variety of connective tissue profiles required to proliferate and assemble SVF EC into tubes.[45]

In addition to connective tissue influencing EC assembly, our own studies with human SVF cultures have shown that the endothelial subfraction can form robust networks in culture in the presence of other stromal cells, and that this process is also mediated by the Wnt signaling pathway, and particularly Wnt5a.[48] While the introduction of a pan-Wnt inhibitor significantly decreased the density of SVF EC networks *in vitro*, we observed that the addition of recombinant Wnt5a could rescue SVF EC density, but not complexity, suggesting that other Wnt isoforms may work with Wnt5a to modulate other aspects of vascular assembly. Subsequent *in vivo* studies echoed our *in vitro* findings, further suggesting that Wnt5a mediates the *in vivo* vascular self-assembly characteristic of SVF. However, the source(s) of Wnt5a within SVF and the precise mechanistic axis by which it acts remain to be investigated.

Because SVF is a diverse population with various progenitor and stem cells, Koh *et al.* sought to determine if the EC in SVF were required to form vascular networks. After depleting EC from freshly isolated SVF, they demonstrated a complete abrogation of SVF vascular network formation *in vivo*.[22] This suggests that the other cell types within SVF are, themselves, insufficient to recapitulate the vascular network formation seen with EC-enriched SVF cells in a defined time period.

SVF EC cells also play critical roles in promoting the survival and functionality of implanted parenchymal cells. We were the first to combine SVF with hepatocytes to form a liver tissue mimic.[6] Using three-dimensional (3D) constructs comprised of SVF and HepG2 hepatocytes, we demonstrated that SVF EC self-assembled into highly intricate cages around HepG2 spheroids. Subsequent tests with a peripherally injected biomolecule (DiI-LDL) showed that the SVF EC vascular cages interacted with the HepG2 clusters, as DiI-LDL localized to these clusters over several hours; to the contrary, constructs lacking SVF were unable to localize DiI-LDL. Thus, SVF EC not only closely interacts and functionalizes parenchymal implants, but also interfaces with the host vasculature such that circulating biomolecules (e.g., DiI-LDL) can make their way toward implanted, metabolically active parenchyma. In a different application, we combined SVF with induced pluripotent stem cell-derived hepatocyte-like cells (iPSC-HLC) and demonstrated that SVF promoted the survival of iPSC-HLC compared with 3D implants devoid of SVF.[7]

In sum, EC are present in all vasculatures and their importance to SVF-derived vascular networks is equally as critical. Additionally, their ability to rapidly self-assemble into networks makes them attractive from a clinical standpoint, as they may offer therapeutic value in settings such as ischemia or with tissue-engineered implants requiring access to the circulation. Yet, it is also important to recognize

that while SVF EC can self-assemble into tube-like structures in the absence of other SVF cells,[45] it is likely the nonendothelial, stromal fraction of SVF confers signals that ultimately shape this network into a mature, functional vasculature. To that end, we present evidence supporting this idea in the following sections.

Adipose stem cells

Zuk *et al.* were the first to show that processed lipoaspirate (PLA) cells could differentiate into one of four lineages—adipogenic, osteogenic, chondrogenic, and myogenic—effectively demonstrating the multipotent potential of adipose tissue.[49] A key parenchymal cell type within adipose tissue is the adipose-derived stromal cells (also known as adipose-derived or adipose stem cells; ASC), which are also considered to be a MSC subpopulation of SVF.[50] In addition to the fact that ASC appear to be immune privileged, these cells have been shown to migrate to sites of injury and release paracrine secretions that reduce inflammation.[51] This trait has been seen with both intraocular injections and tail vein injections, where the former stabilized the progression of streptozotocin-induced diabetic retinopathy[51] and the latter mitigated systemic inflammation, improved glucose tolerance, preserved pancreatic β-cell mass, and increased β-cell proliferation in the face of streptozotocin treatment.[52]

ASC have also been shown to play significant roles with the vasculature. For instance, Cai *et al.* were among the first to demonstrate that ASC impact capillary density and perfusion, and that these actions are mediated by hepatocyte growth factor.[53] When placed with human microvessel EC, ASC improved endothelial network assembly and stability.[54] In trying to determine a mechanism for these outcomes, initial analyses revealed that these cells were positive for a full cadre of markers associated with stromal cells such as mesenchymal cells (CD10, CD13, CD90), pericytes (CD140a/b), and smooth muscle cells (alpha actin, calponin, and caldesmon). Immunohistochemical analysis of ASC-microvessel endothelial cell cocultures showed that after extensive culturing, the cells encased the vascular lumen in much the same way that pericytes did. Again, determining the density of ASC per SVF isolation remains challenging since specific markers have not been identified, although others such as PDGF-B and 3G5[33,38,54–56] and Pref1[57] have been implicated. Additionally, since ASC exhibit variable abundance—white adipose tissue (WAT) is enriched with ASC, for example[50,58]—the sourcing of SVF and its subpopulations needs to be taken into consideration.

To determine a mechanism of interaction between ASC and EC, Merfeld-Clauss *et al.* performed a series of coculture experiments, noting increases in overall extracellular matrix production, ASC expression of alpha-smooth muscle actin, and endothelial expression of CD31.[59] Cocultures yielded the highest degree of endothelial network formation, followed by ASC-conditioned media with the next highest density and ASC fibroblast cocultures with the lowest density. Not only do these findings suggest that ASC endothelial cell interactions are contact dependent (a finding corroborated by Rohringer *et al.*[60]), but also that these interactions progress in both directions (as ASC increased alpha-smooth muscle actin expression as well). This is evidenced in later studies, implicating Activin A as an ASC endothelial cell contact-initiated means of (1) inducing ASC smooth muscle cell differentiation[61] and (2) impairing vascular endothelial growth factor (VEGF) expression, reducing endothelial network formation.[62] In a similar context, Freiman *et al.* cultured ASC progenitors—that is, MSC—with human adipose microvascular EC (HAMEC) in three-dimensional PLLA/PLGA scaffolds.[63] A significantly complex, organized, and mature vasculature resulted after 2 weeks; such findings were supported by the increased expression of alpha-smooth muscle actin as well as increased tube alignment when compared with constructs containing MSC and dermal fibroblasts.

ASC also have the capacity to form vasculatures when interacting with other nonvascular EC types as well. In two separate studies, Strassburg *et al.* discussed the interface between ASC and endothelial cell progenitors (EPC)[64] and lymphatic endothelium[65] as well. The earlier of the two studies demonstrated that a direct interaction between ASC and EPC yielded capillary-like structures in a VEGF-dependent manner—notably, a finding not seen when ASC was cocultured with human EC. With respect to ASC influence on the lymphatic system, Strassburg *et al.* noted that both juxtacrine and paracrine interactions resulted in the increased expression of lymphoid markers and related lymph–vascular architecture. Holnthoner *et al.* described the interaction of ASC with EC isolated from peripheral blood (termed outgrowth endothelial cells) with a 3D fibrin environment, and noted the resulting vascular development and surrounding matrix degradation to be at least partly mediated by matrix metalloproteinase-14 (MMP-14).[66] In totality, these findings further underscore the complex relationship and differences between ASC, progenitor cells, terminally differentiated cells (with particularly interesting findings concerning EPC vs. EC and ASC vs. MSC), and the extracellular environment (such as PLLA/PLGA, collagen, and fibrin scaffolding, for instance). Additionally, the precise mechanism by which the cell populations interact with one another is still a topic undergoing much development.

Even though several of the aforementioned studies assess the interactions between ASC and other ancillary cell types, a key point to emphasize is that ASC are a *subpopulation* within SVF,[33,67–69] and that SVF not only includes ASC, but is also replete with EC, smooth muscle cells, macrophages, and fibroblasts, among others.[6,22,31] The two populations, while related, are distinct and have unique characteristics; key differences between the ASC and SVF are attributable to their postisolation culture. While SVF is the heterogeneous cell pellet seen at the end of a centrifuged, post-enzymatic adipose digestion, ASC are isolated by subjecting SVF to further mixing, lysing, washing, straining, and plating on tissue culture plastic; those cells that are adherent after 72 h in a basic media (such as Dulbecco's modified Eagle's medium [DMEM] with 10% fetal bovine serum [FBS], for instance) are then considered to be ASC.[70] Plated, putative ASC are commonly subcultured to confluence and passaged several times to mitigate any influence by SVF EC and obtain a pure mesenchymal population.[49,71]

Furthermore, like SVF, ASC have demonstrated immense potential as a therapeutic cell source, demonstrating improved outcomes in lung injury,[72] diabetic retinopathy,[73] lymphedema,[74] and vascularization.[59] It should be

recognized that the cellular heterogeneity of SVF is advantageous in that it contains the necessary cells to function as a regenerative therapeutic system. Many of these cell types interact with one another to produce complex vascular and tissue superstructures, and in many of the studies involving ASC, the ASC only yielded its effects when combined with other cell types such as vascular EC[59,62] and lymphoid EC.[65]

Macrophages, monocytes, and immune cells

Adipose SVF contains a significant proportion of cells involved in immunoregulatory[75] and vascular remodeling[22] functions as well. Both functions play critical roles in SVF's ability to self-assemble into a mature vasculature. For example, efforts by Navarro *et al.* demonstrated that adipose monocytes contribute to angiogenesis and even differentiate into EC.[76] These monocyte-derived EC then incorporated into the developing vascular network. Although they concluded that adipose monocytes may constitute a new angiogenic cell source, it is not clear if this monocyte phenotype is typically included in adipose SVF isolations, since others have demonstrated that EC-depleted adipose SVF cannot form vascular networks.[22]

Macrophages have been more extensively characterized in adipose SVF. Morris *et al.* show that $CD11b^+$ macrophages constitute $\sim$20% of rodent adipose SVF isolations.[41] Approximately 80% of these cells were also positive for F4/80, and $\sim$70% were positive for CD301 (a marker for M2 anti-inflammatory, proangiogenic macrophages[75,77–79]). The macrophages from SVF injected intravenously into system circulation were found in the adventitial layer of the saphenous artery, and controlled the artery's vascular tone in an H_2O_2-dependent manner. Thus, it is possible that these macrophages may control tonicity of SVF-derived vessels in a similar manner.

Koh *et al.* also described the role of SVF macrophages on vascular assembly,[22] noting that macrophages were required for proper vascular structural organization. To support this, the depletion of $CD11b^+$ and $F4/80^+$ macrophages from SVF yielded vessels that were blunted and disconnected—findings supported by Fantin *et al.*, who suggested that macrophages facilitate vessel anastomosis.[80] Perhaps more interestingly, they also demonstrated the requirement for an interplay between SVF macrophages and host macrophages. In macrophage-depleted mice, SVF formed endothelial networks with higher vessel integrity at the core than at the periphery. In control mice, the integrity of SVF-derived vessels was uniform. Thus, while SVF can form networks in the absence of macrophages, the quality of these networks appears to critically depend on (1) their presence and (2) synergy between SVF- and host macrophage populations.

There are pathological and therapeutic scenarios that highlight the roles of SVF immune cells. For example, in fat-grafting procedures conducted by Dong *et al.*, the inclusion of SVF in fat grafts lead to increases in CD206 expression (another M2 macrophage phenotype marker), and a downregulation of the proinflammatory agents, IL-1β and IL-6.[35] In another example, rodents given high-fat diets had SVF profiles comprised of a higher proportion of macrophages and monocyte chemotactic protein-1 (MCP-1).[81] In a disease such as obesity, portions of SVF taken from previously obese patients were found to have increased MCP-1 expression[37]; likewise, the hypoxic environment in fat seen with obesity has been shown to increase the amount of SVF-derived inflammatory cytokines that potentiate their effect through a P38-mediated mechanism.[82] Thus, the environment in which SVF cells are situated, that is, in obesity or metabolic syndrome,[83] can have significant effects on their cell proportions (and particularly macrophage subsets) and propensity to incite or propagate inflammation.[77]

SVF isolation and processing

Clinically, SVF can be isolated from discarded human lipoaspirate. Yet, issues to consider include differences in the sites of isolation as well as how the isolated adipose tissue is processed. In addition to regional anatomical differences in fat composition, for instance, the molecular nature of adipose tissue is different with abdominal, medial thigh, lateral breast isolations,[84] and differences exist between brown adipose tissue (BAT) and WAT. Prunet-Marcassus *et al.* demonstrated that the SVF cells isolated from WAT possessed more hematopoietic cells, macrophages, hematopoietic progenitors, and immature cells that, together, contributed to a higher degree of plasticity than SVF cells isolated from BAT.[58]

WAT is not strictly confined to subcutaneous regions. WAT found around the viscera, termed visceral adipose tissue (VAT), are often found in excessive quantities during obesity and metabolic disease. Increases in VAT stores can also raise one's risk of developing cardiovascular disease,[85,86] digestive disorders,[87] and certain urological diseases,[88] to name a few. Given that excessive VAT is detrimental, it is reasonable to ask whether SVF isolated from VAT, compared with SVF from subcutaneous adipose tissue (SAT), is equally as detrimental or dysfunctional. Studies by O'Rourke *et al.* and Benencia *et al.* suggest that VAT SVF promotes inflammation,[82,89] potentially due to a higher proportion of macrophages, natural killer cells, and T cells[82] compared with SVF from SAT. Cohen *et al.* confirmed these findings, showing that SVF isolated from omental WAT or even the serous fluid of the peritoneum were rich in T cells and $CD45^+$ leukocytes, respectively.

Other site-specific variations exist as well. For example, human omental fat was described to be replete with mesothelial cells as opposed to EC,[90] whereas Williams *et al.* describe a predominance of EC in subcutaneous human lipoaspirates.[31] Similar differences can be seen in rodents, in which SVF isolated from inguinal fat pads possessed a higher composition of macrophages and monocytes than SVF derived from epididymal fat stores.[91] Furthermore, inguinal fat is thought to be more plastic and is easier to access.[58] Thus, as EC are the backbone of the developing vasculature, and monocytes and macrophages play significant roles in vascular remodeling during angiogenesis, site-specific differences in SVF composition and plasticity can have profound consequences on the characteristics of the resulting, self-assembled vasculature. These effects should be accounted for during isolation as well as in experimental and clinical applications.

In addition to the differences that site-specific variations can impart on SVF, other considerations have varying effects as well. For instance, the pathological subtype of the

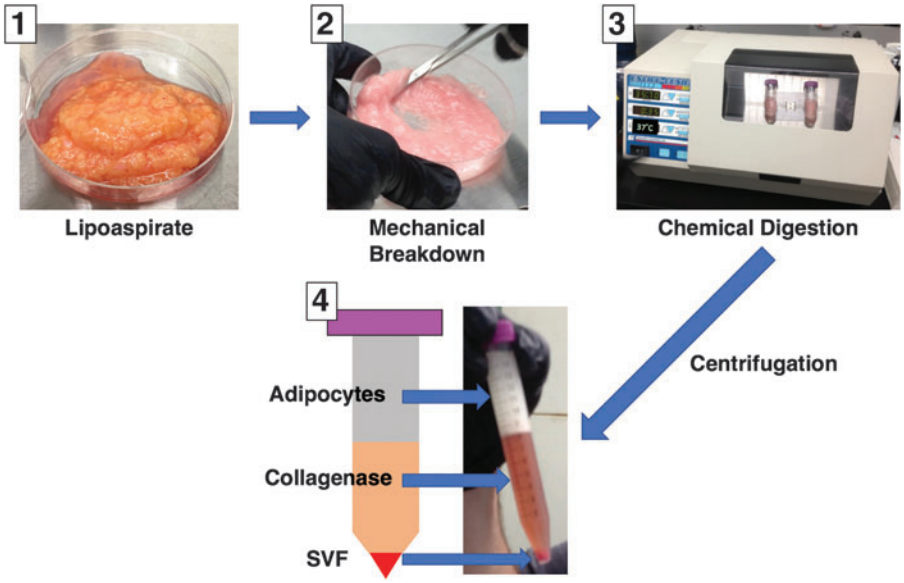

FIG. 2. Manual Processing of lipoaspirate to yield SVF. In Photo 1, lipoaspirate is shown before mechanical breakdown using scissors (Photo 2). This breakdown provides a greater surface area on which the chemical digestion enzyme collagenase can act (Photo 3). After a defined time period of digestion, the resulting slurry is centrifuged, yielding a solution of three key parts as shown in Photo 4: a *top* layer of buoyant adipocytes, a *middle* layer of collagenase and remaining tumescent fluids, and a cellular pellet at the *bottom* containing SVF—a heterogeneous mix of various cell types. Color images available online at www.liebertpub.com/teb

patient (e.g., obesity, smokers, underlying genetic abnormalities, immunocompromised, aging, etc.) can have potentially devastating consequences on native vascular and immunological function,[92–94] let alone the vasculature derived from patient adipose SVF. To that end, there is a relative paucity of high-quality studies clearly defining the impact of pathological disease on the therapeutic potential of SVF, although recent efforts have assessed the role of obesity[37] and aging[95,96] on SVF yields and function.

The isolation modality is another important consideration. Figure 2 depicts a general schematic for how our laboratory manually processes SVF. We, like many others, utilize collagenase for the chemical digestion of lipoaspirate, a technique still considered the gold standard of SVF processing.[97] Still, one potential source of yield or functional variation could lie with the collagenase type, quantity, and/or lot. Williams *et al.* extensively evaluated commercially available brands and lots of collagenase, noting a reduction in collagenase efficacy with purification by dialysis and an enhancement of purified collagenase efficacy by the addition of trypsin.[30] Complementing this, a variety of efforts (also reviewed in Refs.[98,99]) are now focused on the automated or semiautomated isolation of SVF,[97,100–103] many of which eschew chemical digestion and are in clinical trials (e.g., *NCT02234778, NCT01601353,* and *NCT01305863*).

Discussion and Concluding Remarks

The successful generation and survival of large volumes of engineered tissues are likely dependent on the presence of a stable and supportive vascular supply. Host tissues contain parenchyma that can access circulating proteins, hormones, factors, and products through a patent, well-distributed vascular supply. The absence of such a vasculature leads to cell death,[104–108] as oxygen is only capable of diffusing over distances of less than a few hundred microns.[109,110] Additionally, variations in the implant site can impair an engineered parenchyma's ability to interface with existing host vasculatures.[111] An engineered vascular cell support system could serve as an ideal means of meeting the complex metabolic and functional requirements of various engineered parenchyma. One such system is the adipose-derived SVF, which is a heterogeneous mixture of EC, stem cells, macrophages, pericytes, and other vascular support and immunomodulatory cells. Collectively, these cells have functioned to spontaneously self-assemble into vessel-like networks *in vitro*[48,61] and *in vivo* vasculatures that functionally anastomose with the host circulation.[6,22] Such findings suggest that SVF could serve as a therapy for ischemic disease; to this end, preliminary studies have demonstrated that SVF promotes revascularization in peripheral ischemic disease[22,112,113] and myocardial infarction.[114,115]

SVF contains the EC required for vascular network assembly as well as a full complement of support cells that likely play roles in stabilization and maturation.[116–119] Furthermore, SVF may possess the ability to adapt to different clinical scenarios.[120] It is thought to be more plastic than other vascular cell sources, although cell sourcing (e.g., from WAT vs. BAT) can yield site-specific differences.[58] Additionally, SVF contains various populations of stem cells that may facilitate such plasticity and adaptation. In addition to forming a mature vascular network, SVF may also promote healing. Adipose stem cells (ASC) are a MSC subpopulation of SVF[50] that appears to be immune privileged. These cells also migrate to sites of injury[41] and release anti-inflammatory factors.[51]

A third consideration with SVF is that it can be isolated easily, bearing significant clinical and translational implications. Adipose tissue is routinely acquired in a minimally invasive fashion laparoscopically. The isolated adipose tissue is then subjected to enzymatic digestion and processing to yield the SVF. Although SVF isolation can be performed manually, there is a growing interest in automating this process[102,103] for prompt application during an operation or at a patient's bedside.[121] Such automation would enhance repeatability and reduce variability in clinical and experimental outcomes.

In sum, SVF contains a multitude of cell types that act to modulate the immune response, adapt to the host environment, and promote the formation of a robust, self-assembled,

mature vasculature. Its relative ease of isolation makes SVF a clinically relevant, therapeutic cell source that is ideal for numerous applications in tissue engineering.

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