Understanding Adipose-derived Stromal Vascular Fraction (AD-SVF) Cell Biology and Use on the Basis of Cellular, Chemical, Structural and Paracrine Components: A Concise Review

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ABSTRACT

Background: *In the past 10 years, biocellular regenerative medicine has recognized the potential value and availability of a heterogeneous, undifferentiated, nucleated cell population with the adipose tissue complex (ATC). With the recognition of higher mesenchymal stem cell concentrations, ease and safety of access, and the very heterogeneous undifferentiated cell populations in the native 3D adipose matrix, many researchers and clinicians are moving from bone marrow sources to the adipose complex. Approaching the pre-clinical applications from the laboratory state, it is critical to develop an appreciation of the cellular, structural, chemical and paracrine interactions within elements of the stromal vascular fraction (SVF). Further, understanding the importance of platelet-derived growth factors, cytokines, chemokines and adipokines have led clinicians to combine the cell source, native adipose biomatrix (scaffolding), and complex secretory element potential contributions to wound healing and regeneration In vivo. With thousands of clinical experiences in humans in the USA, in vivo, most developing therapeutic modalities are based on clinical outcomes with the majority of reports offering relatively low numbers of cases rather than the longer-term controlled trials often favored. However, with the very high numbers of case reports and small series reporting high safety and efficacy, the results should not be categorically ignored.*

Content: *This paper examines the complex SVF from a cellular, structural, chemical, and mechanical viewpoint. ATC is recognized as the largest endocrine organ in the body which is intrinsically involved in metabolism, immunomodulary activities, and providing an extensive perivascular reservoir of multipotent, undifferentiated cell populations which are involved in homeostasis and regenerative efforts. There is great confusion in use of terms and understanding varied protocols when describing this heterogeneous group of cells and cellular potential functions. As a huge microvascular bed, adipose appears to provide an ideal pool of undifferentiated cells in close proximity to perivascular access for mobilization and relocation demands of injured or diseased structures. Complex signaling mechanisms exist, the understanding of which is gradually forthcoming through a variety of research modalities and application viewpoints. Comparisons of such studies remain challenging, as standardization of protocols remains elusive. The author attempts to bring an understanding of the interface between clinical experiences and the complex mechanisms in play within various microenvironments within the body as understood at the time of writing.*

Discussion: *Understanding the components of the adipose-derived stromal vascular fraction (AD-SVF) is a key to appreciation of the potential uses and contributions in tissue maintenance and repair. Discussion of the concepts of tolerogenesis and regulatory feedback coupled with realization of the microenvironment importance to cellular proliferation and differentiation is presented. The ability to transfer the intact microenvironmental ("niche") components within lipoaspirated samples, with no manipulation, is gradually being recognized as a real potential in the clinical setting. There are potential great advantages to use of non-manipulated tissues from the standpoint of repair potentials, as well as the ability to deliver these elements within a "same surgical procedure" within the practice of medicine. Early research activities were aimed at trying to isolate, concentrate and expand the component cellular groups. We currently believe provision of the entire "smorgasbord" of cells and their native scaffolds (extracellular matrix and perivascular structural tissues) may be of greater value than any one component. Letting the individual site dictate the need through complex signaling and paracrine activities, may be more advantageous than total isolation of elements, particularly in the area of many aesthetic, reconstructive, and musculoskeletal applications. There exists great potential in adipose as the primary source of mesenchymal cells and SVF in biocellular therapies of the future.*

Conclusion: *It appears that interaction of the perivascular elements (AD-SVF and bone marrow-derived stromal fraction) and tissue specific conditions are of major importance for organ and total organism function. Tissue repair, homeostasis, and regenerative capabilities must, by nature, be an inseparable interaction resulting in involvement and coordination of the perivascular complex and each microenvironment it supports. The exponential growth of knowledge and interest in understanding the multiple factors involved in human homeostasis and self-repair characteristics is providing information which will potentially alter many of the existing paradigms currently in the practice of medicine and surgery in the coming years.*

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Introduction

Adipose Tissue Complex (ATC) is intrinsically involved with energy homeostasis, acting as a large scale endocrine organ capable of secretion of a wide variety of regulatory proteins (cytokines, chemokines, and adipokines (such as leptin, adiponectin, and resistin). These elements are involved with many tissue functions, including inflammation, immunity, metabolism, and reproduction. Mature adipocytes comprise the largest volumetric component of adipose tissue, and represent the terminally differentiated cells and are not capable of cellular division per se. Their numbers are maintained via adipogenesis (including proliferation and differentiation) through effects on certain progenitor cells, usually referenced as a progenitor known as the "pre-adipocyte".[1] These cells are considered near-terminal differentiated cells, and 4-8 of which are described as attached to each adipocyte (cell-to-cell), in preparation for the signaling and stimuli for them to continue towards the terminal adipocyte and metabolic function as such.[2]

Zuk, et al in 2001 and 2002 were first to identify the extent and nature of undifferentiated, nucleated cell populations found in adipose tissues.[9, 10] The multipotent nature of these cells focused primarily on the mesenchymal elements within fat as the probable contributor to the multi-lineage capabilities of such tissues to undergo osteogenesis, chondrogenesis and adipogenesis. (*See Diagram 1.*) These findings have been verified extensively, particularly with regard to the mesenchymal stem cell definitions and capabilities. With adipose tissue products readily available as a discard by-product of liposuction procedures, vast quantities of these tissues have come available for study and evaluation. These discoveries sparked extensive clinical and research efforts in providing understanding of how successful autologous fat grafting (AFG) worked from a cellular and subcellular level. It has become clear that the creation of homeostatic-like mechanisms permitted free adipose grafting to be successful, not so much due to mature cell transplantation, but more to the paracrine and undifferentiated cells found within the AD-SVF. Mature adipocytes are now known to be nearly completely lost following AFG, but play an important role as they undergo

For the past 20 years, aesthetic & reconstructive surgeons have safely and effectively utilized autologous fat grafting to provide structural augmentation of the subcutaneous adipose layers and a variety of related tissues. For the first decade, most reports suggested that the goal was to achieve as pure adipocyte cellular graft as possible, isolated with minimal trauma, and transplanted in adipose containing tissues. Microcannulas and closed syringe systems grew in popularity while the observed structural augmentation results improved in predictability and efficacy.[3, 4] Reports of enhanced graft success with the addition of high density platelet concentrates, led to understanding of the potential values of the concentrated growth factors, signal proteins, and cytokines to the acceptance and viability of autologous fat grafting.[5, 6, 7, 8]

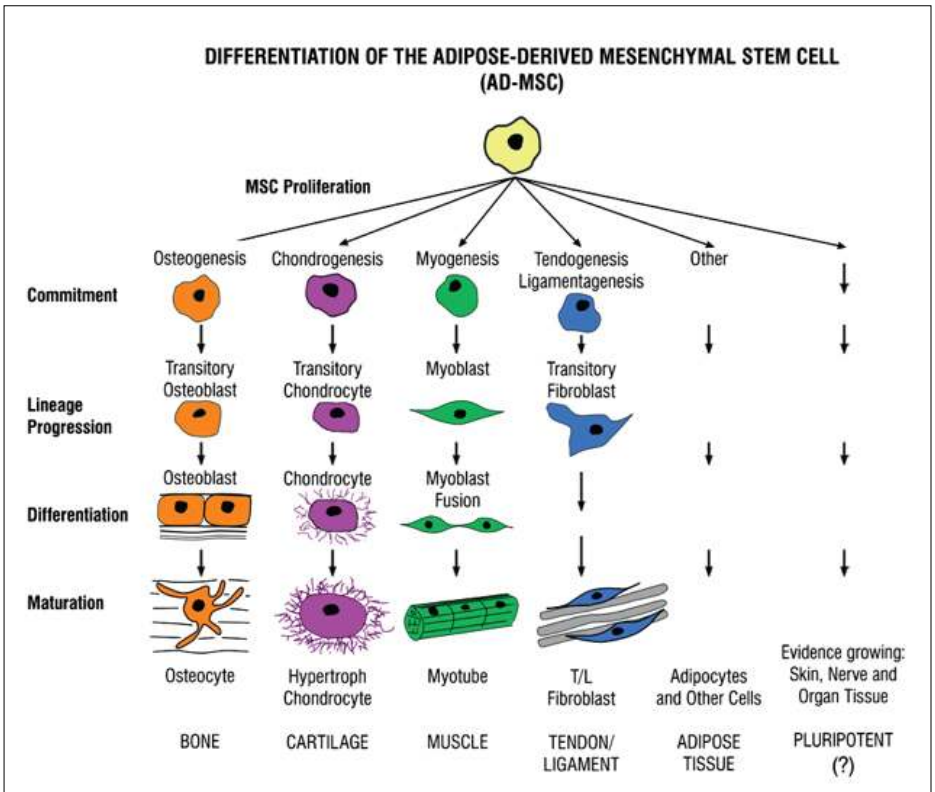

Diagram 1. Flowchart elucidating possible commitment, lineage progression, and maturation of adipose-derived mesenchymal stem cells.
(From Alderman, D., Alexander, R.W., *PPM* Oct. 2011)

degradation following anoxia, by secretion of important protein factors which act to stimulate the attached adipose progenitor cells. This mimics the homeostatic mechanisms utilized by adipocytes as they undergo natural attrition and replenishment in normal cellular lifespans. It is estimated that adipocytes are replaced in a cycle of approximately 5-10% per year, resulting in complete turnover every 5-10 years.[11-14]

Confusion exists amongst researchers and clinicians based on terminology and references to "stem" cells, Mesenchymal stem cells, "adipose stem cells," etc.. For the purposes of understanding this paper, references will be made to adipose-derived stromal vascular fraction (AD-SVF), adipose-derived Mesenchymal stem cells (AD-MSCs), and the adipose-tissue complex (ATC). Accessing the AD-SVF can be made via en bloc resection or lipoaspiration techniques. Differences of these two means of harvesting fundamentally relate to the fact that there is some measured loss of undifferentiated, nucleated cells when examining the total nuclear counts (TNC) and flow cytometry.[15] The nucleated cell composition remains essentially the same, with count differences resulting from the nature and mechanisms of adipose removal via aspiration resulting in lower TNC. This has recently led the aesthetic applications to seek techniques that involve digestion, isolation and concentration of undifferentiated AD-SVF, to be added "back" to the lipoaspirated tissues to restore native cellular quantitative levels. This is termed "cell-assisted" lipoplasty within the aesthetic and reconstructive literature, but not yet permitted in the United States due to regulatory issues.[16, 17]

Appreciation of the multiple potential applications of adipose tissue as a source of undifferentiated cells within the adipose complex has evolved since that time. (*See Figure 1.*) Due to the higher concentrations of MSCs found in the area of the adipose stroma compared to bone marrow sources, many researchers have begun to intensively examine the potentials as adipose offers a less invasive, readily available, and easily accessible resource.[18, 19] At first, the believed ideal goal was to isolate, concentrate, and expand these cells to pure cell-type groups for future reconstructive and regenerative tissue engineering applications. Many animal and human experiments explored the potential protocols to accomplish the isolation and "purification" of cells intended for translation into clinical applications.[20, 21]

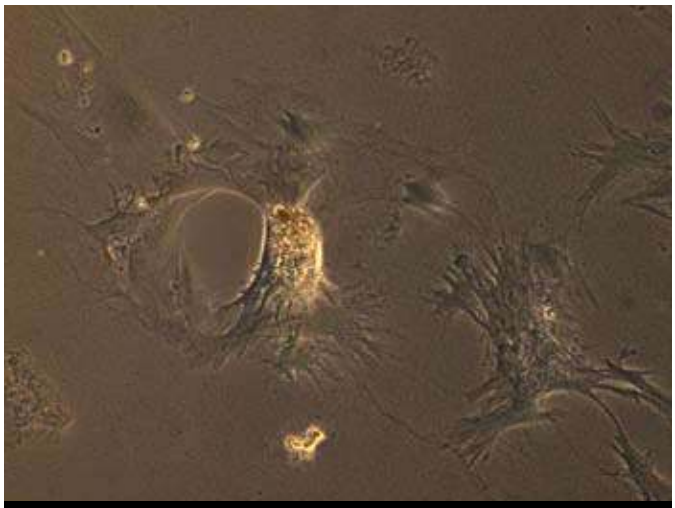

Figure 1. Electron micrograph, adipose-derived nucleated stromal cells. (Adapted From Lyles-Alexander 2004)

Since the mid-late 2000s, many researchers and clinicians began to understand the complexity, contents and paracrine functions of the adipose-derived stromal vascular fraction (AD-SVF). Cellular identification revealed very heterogeneous populations of undifferentiated, mononucleated elements based on cell surface antigens within those multipotent tissues.[22] Chronic inflammatory states are known to effectively reduce stem/stromal cell numbers and proliferative abilities, effectively depleting the available undifferentiated cells and their respective secretive functions needed in healing-regenerative potentials (as in damaged or diseased musculoskeletal tissues). Utilization of non-manipulated adipose complex tissue, coupled with high concentrations of platelet-derived growth factors and signaling protein load, has become an early translator therapeutic modality to treat a variety of musculoskeletal disorders and injuries. Adipose tissue complex (ATC) provides a concentrated SVF and cellular source, plus a native 3-D Bioscaffolding capable of encouraging cellular adhesion and important paracrine functions.[23-26] (*See Figure 2.*)

Attempts to define the ideal use of ATCs have led to appreciation of the myriad of potential effects that can be realized with its use. For example, the *In Vivo* tolerance of grafting the adipose-platelet concentrate combination proved effective, safe and well tolerated in the clinical setting in thousands of structural autologous fat transplantations aesthetic and reconstructive surgery experiences. Further, appreciating the anti-inflammatory and immunomodulary abilities of such tissues have led

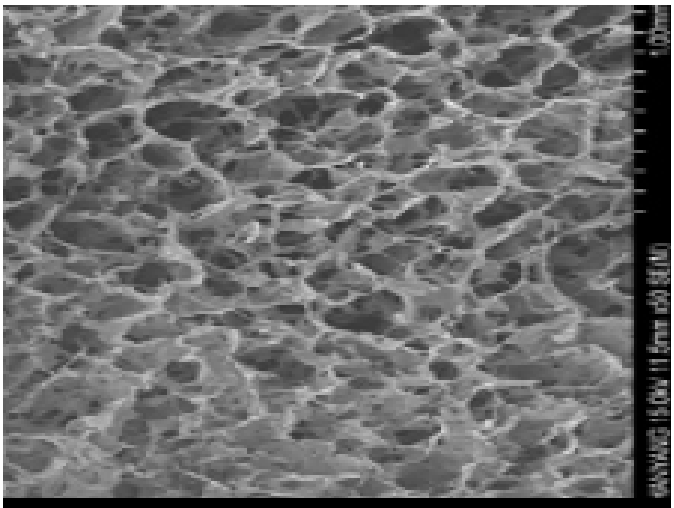

Figure 2. Decellularized, lyophylized native adipose-derived tissue complex (ATC).

researchers to extend uses further into graft versus host modulation with interesting effects. Over the past 7 years in orthopaedic regenerative medicine, the ability to access sterile ATC and provide high-definition ultrasonographic guided adipose tissue transplantation have resulted in the evolution of a recognized clinical procedure of value in a variety of regenerative medical applications, particularly in musculoskeletal and inflammatory disorders.[23, 24] Translating from the proven safety and efficacy of encouraging adipogenesis via placement of small aliquot grafting into native subdermal deposit sites, placement into tendon, ligamentous, muscular, joint and pain sites has been a logical course. Based on the commonality of cellular origins, and the proven abilities to differentiate into a variety of mesodermal related cellular types, it is not surprising that the tissues have responded positively to clinical utilization of such cells and bioactive proteins within the transplant recipient sites for healing and regeneration purposes. This can be accomplished without any chemical manipulation, making it available within the United States, whereas "cell assisted" transfers fall outside that classification as defined by the FDA at the time of this writing.

Understanding the Concept of "Tolerogenesis"

Advancement of the concepts of the "tolerogenic" promotional capabilities of AD-SVF elements were initially published by Ichim et al (2010). Within the complex adipose-derived SVF, elements were reported to be directly capable of promotion of T Regulatory (Treg) cells and, thereby, produce inhibitory effects on macrophages. The concept advanced involved extraction of autologous mononucleated cells naturally contained within AD-SVF, and planned re-administration of these cells systemically. With demonstration of a large number of potent T-regulatory (Treg) cells in AD-SVF, it has been suggested that such may be of significant value in tissues, and may relate to the actual heterogeneous nature and effects of the AD-SVF, promoting not only regenerative properties, but also promote tissue tolerance.[27] Multiple clinical trials are ongoing, including post-infarct remodeling,[28] ischemic heart disease,[29] type I diabetes,[30] and liver failure.[31] There are ongoing studies to delineate similar effects when non-isolated or concentrated SVF cellular-matrix groups are placed within select tissue niches.[32]

The therapeutic rationale for use of these cells and the high content of native multipotent MSCs within the AD-SVF has gained substantial clinical interest. In a very well referenced review and study, Ichim et al (2010) suggested that AD-SVF may offer very important contributions to reduction of inflammation, and favoring re-equilibration of tolergenic mechanisms. Their examples utilized rheumatoid arthritis as a form of autoimmune disorder in which induction tolerance is sought, as well as simultaneous induction of tissue regeneration.[27] In addition to ongoing clinical applications and experiential trials, thousands of guided injection therapeutic treatments of adipose-derived stroma and its biomatrix, plus HD PRP concentrates, are being done each year with excellent safety and success. Most of these treatments are reported as case reports, small series or multiple case reports, without ongoing multicentric randomized formats completed at this time. The sheer numbers of experiences, however, cannot be ignored during the translational uses in musculoskeletal cases, particularly as standard protocols are evolving to measure outcomes and commonly accepted metrics. Gathering of all data with comparable metrics and outcomes should be a high priority goal within all organizations performing autologous fat grafting utilization.

This review is intended to provide a concise review of the current understanding of the importance and nature of the adipose complex, particularly within its AD-SVF.

Adipose-Derived Stromal Vascular Fraction Main Components

Within the AD-SVFis a collection of heterogeneous cells and components, most notably: MSC, HSC, Treg Cells, Pericyte-EC, mast-cells, complex microvascular beds (fibroblasts, WBC, dendritic cells, intra-adventitial smooth muscular-like cells, etc.), and extracellular matrix.[33, 34, 35] In addition, complex adipose tissue (ATC) harvested via en bloc excision or lipoaspiration, delivers mature adipocytes and their attached progenitor cells (pre-adipocytes of presumed unipotent abilities as near terminally differentiated cells) which represent another group of secretory-capable cells within the grafted microenvironment they accompany in transplantation procedures. Appreciation of the importance of the complex, 3-dimensional matrix found in adipose tissues have led many to consider the intact, non-manipulated transplantation of such tissues and their accompanying cellular elements may be advantageous.[36] (*See Diagram 2.*)

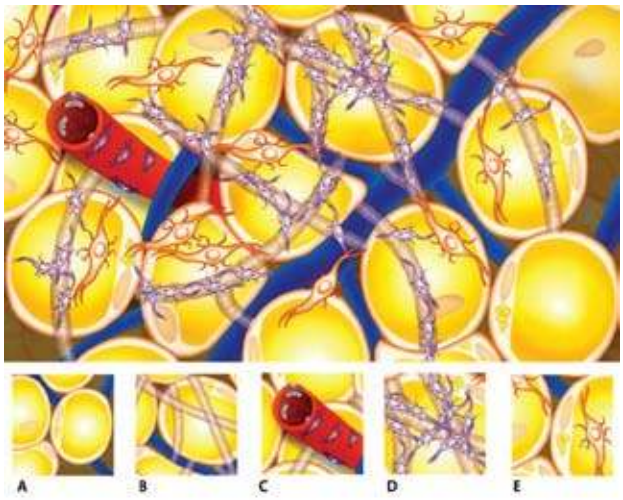

Diagram 2. A. Terminally differentiated mature adipocytes. **B.** Extracellular matrix (fibroblastic elements, most nucleated, undifferentiated cells adherent to ECM & Heterogeneous Perivascular Cells (Cell-To-Cell contacts). **C.** Pericytes-Endothelial Within Microvascular Elements (important in angiogenesis). **D.** Mesenchymal stem cells (Approximately 45% of nucleated cell counts in SVF). **E.** Pre-adipocyte (progenitor cells, near terminal differentiated form, adherent to adipocytes).
(Adapted From Alderman, D., Alexander, R.W.: *JOP* (2011) Vol. 3)

In most experimental studies, the cellular elements within AD-SVF is extracted and separated by application of protocols similar to or developed by Zuk et al.[9, 10] Characterizations of the cell subpopulations constituting the AD-SVF often revolves around the examination of CD 34^+ cells, which represent 50-80% of the group. Difficulties in comparative studies typically result from the use of a varied panel of antigens. Hematopoietic components are often studied based on colony-forming assay (CFU-F) for quantification purposes. The endothelial cell fraction is often investigated by quantification of co-expression of several endothelial markers and *Ulex europaeus* agglutinin 1 (UEA-1) binding. The majority of CD 34^+ are found widely distributed among adipose tissues, predominantly associated with vascular and ECM structures. From freshly isolated AD-SVF, the majority of CD 34^+ are found to be CD 31^-/CD 144^-, and easily separated from a distinct population of CD 34^+/CD 31^-/CD 144^- (endothelial cell markers) by differential attachment on uncoated plastic, suggesting that the non-endothelial population occupy a pericytic position. Traktuev et al, analyzed surface and intracellular markers of freshly isolated CD 34^+/CD 31^-/CD 144^- and reported that >90% coexpress CD 10, CD 13, and CD 90, pericytic (chondroitin sulfate proteoglycan, CD 140a and CD 140b), and smooth muscle (alpha-actin, caldesmon, and calponin) markers. In co-culture of AD-SVF cells with human endothelial cells suggested a bi-directional paracrine interaction between these cells.[37] (*See Figures 3 & 4.*) This information suggests that the majority of AD-SVF adherent CD 34^+ cells may represent resident pericytes who play a role in vascular stabilization by mutual structural and functional interactions with endothelial cells.

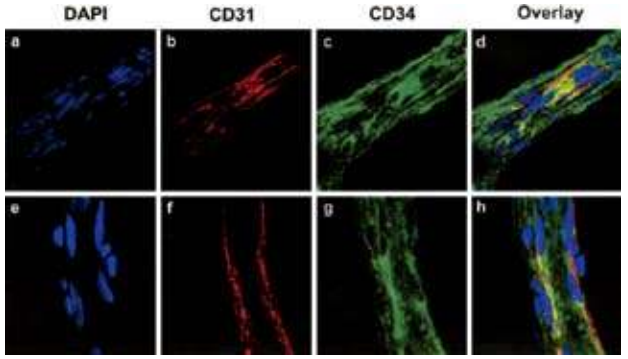

Figure 3. Histological analysis of human adipose tissue. Frozen sections of human fat stained for the endothelial marker CD31 (red) and a major fresh ASC marker CD34 (green). Nuclei are revealed by 4',6-diamidino-2-phenylindole (DAPI) staining.
(Traktuev, D. et al: *Circ Res* 2008)

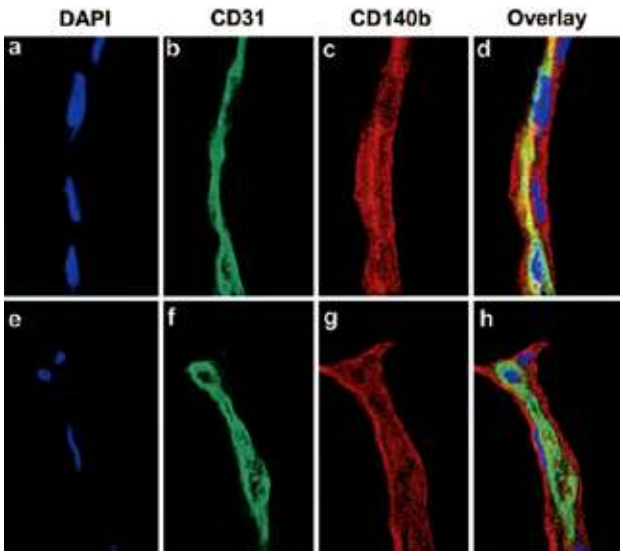

Figure 4. Histological analysis of human adipose tissue for microvascular endothelium and CD140b-expressing cells. Frozen sections of human fat were stained against endothelial CD31 (green) and ASC/pericyte CD140b (red) markers. Nuclei are revealed by DAPI staining. (Traktuev, D. et al: *Circ Res* 2008)

Bidirectional paracrine interaction is supported by identification of angiogenic factors (VEGF, HFG, bFGF), inflammatory factors (IL-6 and IL-8, monocyte chemoattractant protein-1 and -2), mobilization factors (macrophage colony-stimulating factor and granulocyte/macrophage colony-stimulating factor) in media conditioned for CD 34^+ from adipose-derived stromal cells. Traktuev et al also reported an active and robust mitogenic response when these cells were exposed to basic-FGF, EGF, PDGF-bb. These are prevalent in endothelial cells, plus found in HD PRP concentrate secretion from alpha granules. This finding may offer support to the potential advantages offered to the combination of AD-SVF when exposed to HD PRP in autologous tissue transplantations.

Appreciation of some of the adherent undifferentiated cells found within the living bioscaffold comprising the structural aspect of the adipose tissue complex (ATC) is gaining importance along with better identification of component parts. Gradual return to the concept that it may be more important to provide sites of disease or injury with a full selection of multipotent cells and secretory elements has been increasing in the past two years.[38] Maumas et al, concluded that adipose-derived stromal cells display specific characteristics. They are: 1). CD 34^+ (expression of which is reduced *In Vitro*) when cells proliferate in culture); 2). Display both stromal and perivascular positions; 3). Exhibit (*In Situ*) an unusual morphology resembling dendritic protrusions; 4). Do not express some specific pericyte markers (such as NG2, CD 140b, or alpha-smooth muscle actin (alpha-SMA); and, 5). Adipose stromal seems to support adipose tissue growth in obesity (adipocyte hyperplasia), as their numbers are decreased and adipogenic markers increased in obesity.[39]

1. ADIPOSE-DERIVED MESENCHYMAL STEM/STROMAL CELLS (AD-MSCS)

AD-MSCs are considered a significant nucleated cell population, of high value within ATC, as fat tissue has, proportionally, a very high concentration of these cells compared with other tissues, particularly bone marrow.[40] This concentration is considered by many to be high enough to stimulate many regenerative needs without requirement of major manipulation in *Ex Vivo* isolation and expansion. (*See Figure 5.*)

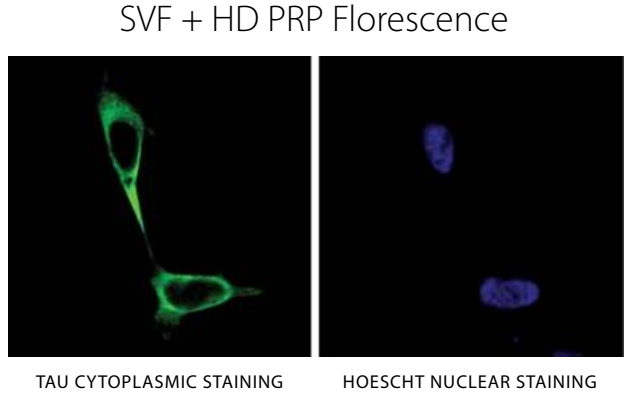

Figure 5. Adipose-Derived Mesenchymal Stromal Cells.

MSCs represents a cellular population which offers immune modulatory adherent cells which are capable of differentiation into bone, cartilage and adipose tissues and several other tissues.[27] They are found in many tissues, including bone,[9, 10, 20] adipose,[41] heart,[42] Whartons Jelly,[43] dental pulp,[44] peripheral blood,[45] umbilical cord blood[46] and menstrual blood.[47] These cells show remarkable similarities in cellular capabilities, in addition to their homeostatic, growth and regenerative roles. They possess certain anti-inflammatory properties which appear to suppress inflammation through secretion of mediators including IL-10,[48] IL-17,[49] TGF-B superfamily,[50] LIF,[51] soluble HLA-G,[52] and IL-1 receptor antagonist.[53] Expression of immune regulatory enzymes such as cyclooxygenase,[54] and, indolamine 2,3 deoxygenase[55]

are involved in the tolerogenic processes. Indirectly, they may inhibit autoimmunity through the ability to induce generation of T Regulatory cells (Treg).[56]

Regeneration of diseased or injured tissue from MSCs (as a cell source), coupled with the important anti-inflammatory effects, have been reported in a variety of immune and inflammatory pathologies including MS,[57] GVHD (graft versus host responses),[58-63] SLE (systemic lupus erythematosis),[64] and end-stage liver disease.[65] This combination is an important basis for belief that MSCs may play a significant role in reconstructive and regenerative therapies in the future.

2. HEMATOPOIETIC STEM CELLS (HSCS) WITHIN ADIPOSE SVF

Defined as $CD34^+$ stem cells of hematopoietic origin, HSCs have been extensively studies for many years, and are being used in clinical therapies. Initially such $CD34^+$ cells were thought to be of prime importance to provide transdifferentiation actions leading to replacement of damaged tissue elements. Researchers now believe that many of the trophic signals and repair may be due to paracrine functions rather than cellular contributions. HSCs have been reported to express many growth factors, including VEFG, HGF, IGF-1, and FGF-2. Besides local changes, an important contribution to angiogenesis (functional collateralization) is considered of significant value. This has led to studies in critical limb ischemia and induced hindlimb models as a therapeutic aid.

HSC also display direct immune suppressive and tolerance inducing ability.[66] The ability to deplete reactive T cells[67] may be involved. As is further discussed in this review, TFG- B and the TGF superfamily of cytokines are considered important effectors for the Treg suppression of such reactive T cells,[68] neutrophils,[69] macrophages,[70] and dendritic cells (DC). These findings have been implicated as an autocrine factor released by CD34+. Massberg et al (2007) suggested an "Immunosurveillance" role for circulating CD34+ cells in circulation via activation and differentiation of these cells into dendritic cells (DC) via of toll-like receptors (TLR) agonists.[71]

3. T REGULATORY CELLS (TREG) & ALTERNATIVELY ACTIVATED MONOCYTES

Adipose tissues possess increased numbers of Treg Cells based on functionality and expression of $CD4^+$, $CD25^+$, and $FoxP3^+$ phenotypes.[72] Besides the Treg suppressive function of reactive T cells, adipose-derived Treg cells seem to be enhanced in tissue culture, with increased secretion of leptin and TNF-alpha which may inhibit Treg proliferation and activity *In vivo*.[73, 74] This is of potential value in attaining tolerance within an ongoing immune response.[75] SVF in immune modulation may also be of value based on the expression of high numbers of alternatively activated macrophages.[76]

4. ADIPOCYTES & ATTACHED PROGENITOR CELLS

Adipocytes that are transplanted within aesthetic and reconstructive procedures were thought to be of paramount importance when trying to encourage structural restoration of lost tissue fullness.[77] It is becoming increasingly clear that the mature adipocytes transferred, having undergone a complete anoxic period, are gradually lost within the first few days following transfer[78] This is not suggesting that those adipocytes are not important in the ultimate result of structural improvement, as they gradually are lost, important signals are secreted to stimulate niche progenitor cells attached to each adipocyte to become metabolically active and provide replacement immature adipocytes. This further supports the hypothesis that transfer of mature cells and the concomitant mononucleated cell population are important to the microenvironment into which they are grafted. It is well established that adipose grafted to adipose tissue favors adipogenesis, felt to be based on the important natural 3-dimensional microenvironment of the ATC.[79]

Activation and proliferation of multipotent cells within the graft and the recipient site seems to work synergistically to produce a regenerative function, while preserving immunologic integrity and providing important anti-inflammatory and associated cellular responses. This appears to be a very important tolerance induction function, which may translate to future applications where cellular regenerative tissue needs can be combined with local area inflammatory controls favoring healing and regenerative activities.

5. PERIVASCULAR CELLULAR COMPONENTS

Appreciating the versatility and multipotent potentials of the stromal vascular fraction (SVF) cells has been enhanced as the extracellular matrix (ECM) has become an important factor in development of translational

uses of non-manipulated adipose tissue complex (ATC). Understanding the microenvironment (niche) physical and chemical controls of undifferentiated cellular elements inevitably leads us to appreciate the importance and magnitude of the microvascular structures in adipose tissues. As such, the complex vascular organ presence in all tissues except cartilage and cornea, may point to the basic adult undifferentiated cellular complex including pericytes, endothelial cells (EC), and mural smooth muscular cells. Some believe that these cells may, in fact, be the precursor source for the mesenchymal stem cells per se.[80-82]

Zimmerlin et al (2010) developed a multiparameter flow cytometric analysis and sorting strategy which identified four distinct vessel-associated cell populations within the non-hematopoietic SVF by three antigen markers (CD31, CD34, 146 CD). They described two "mesenchymal" and two "pericyte-endothelial" populations, which were further defined in immune-histological analysis by location. It was pointed out that, use of simplified separation strategies may result in masking of the true heterogeneity of the AD-SVF resulting in lumping multiple cell populations occupying discrete anatomical positions within the microenvironments. This fact may contribute to the widely variant reporting of the components and functions attributed to the complex SVF in adipose tissues.[83]

The potential regenerative and therapeutic value of these cells is rapidly being recognized, due to ubiquitous distribution and concentrations of perivascular elements representing pools of adult stem/stromal cells ready to be recruited and committed in response to a variety of chemical and physical demands. Areas of multipotent cells are often under the regulation of the transforming growth factor (TGF-B) superfamily. Regulation of such pools effects cellular activation, differentiation, recruitment and organization within the various host tissues and includes complex feedback loops involving cell-to-cell and secretory elements. TGF-B, plus a variety of growth factors and cytokines are known to act upon the microenvironment of a wide variety of tissues, and thought to have a direct bearing on the cellular differentiation selected within a tissue niche, and on homing abilities to recruit needed cellular elements to target sites of need.[83]

As the vasculature is a constantly changing and adapting tissue throughout the body, it seems logical to concentrate stem cells in contact with the SVF would be an ideal distribution interface for rapid response to injury and disease, as well as to maintain homeostatic mechanisms throughout the body.

Common vascular disorders, such as atherosclerosis, display a variety of ectopic tissue formations, such as bone, cartilage, fat, and bone marrow stromal-like deposits within arterial walls.[84] This suggests the participation of multipotent cells within the vascular tissue complex may be involved in recruitment of other ectopic tissue precursors, triggered by injury, damage or inflammatory conditions.[85] The changes may represent direct perivascular stem cell stimulation, or to recruitment from distant undifferentiated niche cells, which are "targeted" to "home" and engraft on the site of damage or disease. Regardless, such "stem cell effects" are thought to favor certain cell lineages and types to be under feedback controls which affect the perivascular and vascular system as a target tissue.[86]

When dealing with biologic tissue changes, understanding the cross-talk (feedback loops) of cell populations, occasional de-differentiation of cells and their physical organization, and coordination within microenvironments may prove very pertinent to regeneration, function, or homeostasis in most or all tissues. An example of this is clear within the adipose tissue complex itself. When fat is grafted from one area of the body and transplanted into an adipose native environment, there is a very strong and direct interaction that favors the transplanted adipocytes, their attached precursor cells,[87] and SVF to be directed towards adipogenesis with a strong angiogenic stimulation. When non-manipulated adipose tissues are transplanted into skeletal muscle, ligament, and tendon sites, the adipocytes degenerate while expressing significant signal proteins and growth factors which appear to act upon local resident tissue cells and undifferentiated components from the graft and its SVF elements. This seems to confirm the ability of "site-specific" controls of undifferentiated elements to favor cell lineages needed at the site of damage or disease. This is the basis for the emergence of translational biocellular regenerative therapeutic treatments utilizing adipose-derived stem/stromal cells in the musculoskeletal applications.[88] The detailed mechanisms of these changes remain the subject of ongoing clinical trials and laboratory study.

Members of the TGF-B superfamily have been implicated in many regulatory functions, and are known to have specific functions within the vascular tissue complex.

For example, bone morphogenic proteins (BMPs) are known to support undifferentiated cell renewal,[89, 90] favor certain vascular lineage differentiation, and encourage the structural design within the perivascular and vascular elements.[91-93] Luo, G et al reported spontaneous calcifications and cartilage formation within arterial walls of matrix Gla protein (MGP) knockout mouse, a known antagonist of BMPs.[94] Yao et al also reported AV malformations in the presence of BMPs inhibition via matrix Gla protein. In addition, they demonstrated that inhibition of BMPs provides protection against such calcifications and atherosclerosis.[95] The importance of these observations may verify the significant role of the relationships between perivascular tissues and site-specific cell interactions. Understanding these mechanisms is gradually evolving, and may have a critical contribution to optimizing regenerative therapeutic applications.

Regulatory Feedback Within Adult Stem-Stromal Cells

Due to spatial limitations in many tissues, distribution of adult stem cells would seem potentially problematic. Close relationships between the SVF and ECM in tissues would serve to provide a variable mechanism by which our tissues are able to maintain a concentrated and readily available pool of undifferentiated cells. This permits wide area distribution which is advantageous when injury or dysfunction occurs which make them readily available for rapid proliferation, differentiation, and mobilization within tissues. As SVF tissues offer cell sources for differentiation plus the angiogenic capabilities of vascular response combined, it makes the concentrations of such cellular and paracrine interactions well suited to respond to needs within local microenvironments.[96, 97]

The importance of understanding microenvironment (niches) is a common challenge in evaluating stem cells or "in-transit" cells,[96] all bearing a close relationship to the basement membranes in tissues. Watt and Hogan described vascular microenvironments being located in proximity of endothelium, which could modulate behaviors of pericyte/ECs via growth factors, signal proteins, and vascular matrix elements. By concentrating undifferentiated cells in such areas could promote rapid access to needed cells and facilitating more rapid tissue regenerative properties.[98] Research has demonstrated that mesenchymal stem cells, including pericytes, so-called calcifying vascular cells, and smooth muscle cells are capable of osteo-chondrogenic differentiation spontaneously or in cases of hyperphosphatemia.[99] In addition to locally available cells, circulating undifferentiated cells and marrow elements may provide additional access to multipotent cells populations.[100]

Lander[96, 97] effectively described that stem-stromal cells pass through a number of transitory or intermediary steps before reaching terminal differentiation. These cells, during transition, may react to activated growth factors and signal proteins to provide feedback control within the microenvironment. On that basis, it may be that such feedback would serve as both a stem cell control as well as affecting those transitory cells to accelerate more rapid regeneration after injury or disease.

Negative feedback secretions also can control proliferation within a niche, and include members of the TFG-B superfamily. For example, locally derived BMP agonists and BMP antagonists are felt to be important and available to provide regulatory functions within populations of precursor cells.[89, 90]

Is Stem-Stromal Cell Differentiation Purely a Function of Cell Type?

Defining a stem-stromal cell, or what it is specifically designated to do, is not clear cut at this time. Lander (2009) suggests that definition of stem cells should be held in relation to context and condition, and not based on specific cell descriptors.[97] The entire controversy surrounding "stemness" is based on poorly defined characteristics which delineate undifferentiated cells from terminally (or near-terminal) differentiated cells in tissues. This is further compounded by the alterations of cells under the influence of diverse growth factors, cytokines/chemokines and the variety of recognized inhibitors which offer some insight as to the actions of the heterogeneous group of nucleated multipotent cells found in the SVF of most tissues. Evidence is accumulating that some stem-stromal cells may be able to "de-differentiate" under certain conditions, and that such alterations may be contributory to some disease states.[101]

As example, transdifferentiation has been suggested as a potential contributory mechanism to certain osteogenic changes resulting in ectopic calcifications such as seen in progressive fibrodysplasia ossificans.[102] Genetic mutation for the activin-like kinase receptor (ALK)2, which is a BMP-1 receptor, and which is activated to cause alteration to osteogenic lineages and calcification within soft tissues. Similar mechanisms are thought to play a role in atherosclerosis and intravascular calcifications through BMP signal pathways.[95]

Multipotent mesenchymal stem cells have been shown to exhibit certain structural patterns *In vitro*, and which may be important in microenvironment changes in various situations. These cells generate a pattern of ridges and nodules[103, 104] in which bone formation occurs. As these structural patterns have highly concentrated BMPs and inhibitors, effects may be seen in facilitation of vascular response and angiogenesis commonly reported in transfer of adipose-derived stromal elements into musculoskeletal and structural adipose tissues.[105, 106]

How Does Vasculature Coordinate with Tissue Specific Functions?

Complex interactions, that are now not completely understood, are necessary for coordination of tissue specific demands and supportive angiogenesis. Structural demands and stresses of any tissue type must coordinate with the vascular capabilities needed to support the metabolic and functions within such tissues. An example of this coordination is reflected in radical weight changes, with the vascular tissues ability to continually expand and contract to those changes within the adipose tissues (as in obesity). Effective tissue regeneration must include the interactions for growth and organ-specific cellular responses in order to maintain homeostasis and organ function. It is not yet understood how specific signaling and exposure to target tissue results in differences of cell lineages considering both the microenvironment and SVF cells are simultaneously exposed. It is clear that the ability of the vascular tissue within the adipose complex to expand and contract in conjunction with overall body weight gain and loss. This seems to favor the body's demonstration of the ability to adapt, *In vivo*, to tissue demands. Recognition that obesity is a form of inflammatory disease, and as such, provides signaling for changing vascular patterns, have offered an explanation of the SVF importance in adipogenesis.[107]

Yao et al, suggests a potential mechanism in reporting that endothelial cells (EC) and type II lung epithelial cells respond in opposite fashion to the same stimulus.[108] In this example, EC proliferation was stimulated by BMP-4 mediated by VEFG, whereas lung epithelial cells were suppressed. When BMP-4 activity was inhibited, the lung epithelial cells were stimulated. They suggested that "reciprocal regulation" was expressed, *In vivo*, in the balance of airway function and vascularity in the lungs of MGP transgenic mice. Airway formation was found to be dominant in MGP mice, with vascular proliferation dominate in MGP-deficient mice. They concluded that since optimal gas exchange in the lungs are linked directly to the proliferative response of the two different groups of cells, reciprocation of two adjacent cellular types is essential to organ function. The mechanisms responsible for such regulatory functions remain unclear.

Lander et al (2009) looked at the actions of many tissues and organs to grow to precise sizes and configurations, and when injured, promoted regenerative healing abilities in an accurate and timely fashion. By mathematical modeling, they examined whether organization of cells into specific lineages and feedback interactions, are the active elements in tissue control strategies. They reported that tissue performance objectives can be simultaneously achieved through a combination of lineage structures, signaling mechanisms, and spatial distributions of cell types that correspond with observed regeneration within tissues (repair and homeostasis). The key to successful control is described as an "integral-feedback mechanism", implemented when terminally differentiated cells secrete molecules which lower the probability that progenitor cells replicate versus differentiate. They concluded that this mechanism explains how proliferative behaviors of undifferentiated cells and "transit-amplifying" cell populations can emerge as a consequence of feedback mechanisms, rather than intrinsic programming of cell types.[109]

Adipose tissue represents a very complex association of cellular components. It is a mix of mature adipocytes, progenitor cells attached to adipocytes (pre-adipocyte), mesenchymal stem cells, ECM, SVF and a heterogeneous

nucleated cell population (which includes pericytes/EC, mural smooth muscle cells, and white blood cells). At this time, it is impossible to specifically define the cell-to-cell and cell-to-matrix interactions, or the complex autocrine-paracrine factors within the microenvironment. Several researchers have suggested that the perivascular cell components may, in fact, be the "original" stem cell providing mesenchymal stem cells to a wide variety of tissues.[80, 110] It seems that the SVF located in great supply within adipose tissue microvasculature may provide a cellular "smorgasbord" of undifferentiated cells which can be activated via complex physical and chemical interactions within essentially all tissues. This, in fact, is the premise for reports of non-manipulated adipose tissue complex mixed with HD PRP concentrates in regenerative applications in musculoskeletal and a variety of therapies evolving.[4, 23-25] Co-stimulatory effects of platelet-derived growth factors, cytokines/chemokines, combined with secreted elements enhanced with VEGF within the tissues, is directly involved in stimulating the angiogenic responses essential to tissue nurturing and survival.[79] Reports in aesthetic-reconstructive surgery and regenerative therapy in musculoskeletal applications confirm the contribution of this combination of stem-stromal cells, including the importance of native complex 3-dimensional native scaffolding within ATC, and use with high-density platelet-rich plasma concentrates (HD PRP) defined at >4-6X measured patient baseline levels) has proven safe and successful in treating human subjects. From structural augmentation, improvement of hypertrophic or post-radiation scarring, wound healing, to orthopedic regenerative therapy, each use is strongly associated with cellular change and angiogenic-vascular responses.[109]

Reports of importance of these coordinated elements are also found in neurological disturbances. The neurovascular network, coupled with the available undifferentiated cell populations associated with the AD-SVF likely have important contributions to homeostasis and repair of neurological damage or disease. It is possible that perivascular stromal cells and neurons may respond to similar molecular stimuli or may act as a co-regulator of homing to an injury site. Vascular signals such as VEGF and Notch have been reported as important in cellular fate in both the nervous and vascular systems.[110] This association may be integral to neurogenesis processes and maintaining/repairing neurological function.

Researchers have extensively studied the component elements within AD-SVF *In vitro* analyses, but recognize the inability to translate from 2D tissue culture conditions and manipulations, to the complex 3-D microenvironments encountered *In Vivo*. Although important to carefully analyze the isolated behaviors of the undifferentiated cells within the body, gradual beliefs are emerging that, rather than isolate/concentrate for clinical applications, it may be of more value to permit the individual microenvironment to dictate the interactions and utilization. This "smorgasbord" approach to translational therapies is best illustrated by the thousands of guided injections of non-manipulated AD-SVF and bone marrow-derived stromal fraction in musculoskeletal applications. Successes are being reported on case-by-case reporting, pending the extensive controlled clinical trials started or soon to begin. Rather than practitioners attempting to identify the "most" important cell types or paracrine elements needed to accomplish wound repair and tissue regeneration, that leaving those processes to be dictated by diseased or injured sites may be much more effective. At this point in time, extensive research is required to better understand the component parts involved, but replication of the true microenvironments of all tissues *In vitro* remain somewhat outside our current capabilities or understanding.

Potential for AD-MSCs and SVF in "Biocellular" Therapy

Use of AD-SVF and its components in biocellular strategies may offer significant potential advantages compared to external medications and/or practitioner selected biological cell treatments. Besides the intrinsic value of the native bioscaffolding within complex adipose tissues, the heterogeneous group of attached cells available for site-specific selection seems to offer great potential in regenerative medicine and surgery. In particular, the MSC components alone offer some substantial contribution abilities such as: a). Local site immune modulation via production of anti-inflammatory factors (TGF-B, HLA-G, IL-10, neuropilan-a ligands and semiphorin-3A[113, 114]; b). Potential to home to injury sites locally; and, c). Ability to regenerate diseased or injured cells via direct transdifferentiation influenced by local microenvironment cells and a variety of secreted factors.

Importance of Additional Research and Reporting

As we are currently in an exponentially growing translational period for use of adult stem/stromal cells, the importance of completing standardization of protocols and clinical studies becomes vastly greater. The scientific understanding supportive of these early clinical uses and observations is rapidly evolving. Large sample, controlled clinical trials are essential to establish the true potential of adult adipose-derived stem-stromal cells. At the time of this writing, more than 80 such studies are underway or in the recruitment phase. (www.clinicaltrials.gov: Keywords - Adipose Stromal Vascular Fraction; Mesenchymal Stem Cells; Adipose Stem Cells; Adult Stem Cells Stromal Vascular Fraction; Adult Stem Cells).

Conclusions

There appears to be an inseparable coordination between tissue-specific elements and the supportive AD-SVF and vascular components. Although the details of this complex process remains incompletely understood, such a relationship appears essential to normal organ and tissue homeostasis and repair mechanisms *In vivo*. The interactions and cooperation appear to have significant importance in the development, maintenance, and regeneration abilities of the body. Further, loss of such regulation and cooperation may be intrinsically involved in disease or degenerative processes.

A goal in aesthetic, reconstructive, and regenerative patient care is to identify the component parts and identify how undifferentiated cells and AD-SVF can be directed, *In vivo*, to areas of dysfunction or degeneration, as well as understand our homeostatic mechanisms. This information is essential for us to be able to optimize or promote specific stem cell activity and lineage differentiation processes in future regenerative medical applications.

It appears that the interaction of perivascular elements (AD-SVF and BM-SVF) and tissue specific conditions are of major importance for organ and total organism function. Tissue repair, homeostasis, and regenerative capabilities must, by nature, be an inseparable interaction resulting in involvement and coordination of the perivascular complex and each microenvironment it supports.

The exponential growth of knowledge and interest in understanding the multiple factors involved in human homeostasis and self-repair characteristics is providing information which will potentially alter many of the existing paradigms currently in the practice of medicine and surgery in the coming years. ■

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