Adipose-Derived Stem Cells for Regenerative Medicine

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This Review is part of a thematic series on the **Pathobiology of Obesity**, which includes the following articles:

Adipose-Derived Stem Cells for Regenerative Medicine

Obesity: Energy Metabolism at the Heart of the Problem

Lipid Disorders and the Metabolic Syndrome

Obesity and Leptin Resistance

Adiponectin as a Cardiovascular Protectant

Gary Lopaschuk, Guest Editor

Adipose-Derived Stem Cells for Regenerative Medicine

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Abstract—The emerging field of regenerative medicine will require a reliable source of stem cells in addition to biomaterial scaffolds and cytokine growth factors. Adipose tissue represents an abundant and accessible source of adult stem cells with the ability to differentiate along multiple lineage pathways. The isolation, characterization, and preclinical and clinical application of adipose-derived stem cells (ASCs) are reviewed in this article. **(*Circ Res*. 2007;100:1249-1260.)**

Key Words: adipose tissue ■ adult stem cells ■ bone marrow stromal cell ■ differentiation ■ tissue engineering

Tissue engineering and regenerative medicine is a multidisciplinary science that has evolved in parallel with recent biotechnological advances. It combines biomaterials, growth factors, and stem cells to repair failing organs.[1] Material scientists can now fabricate biocompatible scaffolds with a wide range of physical parameters, combining mechanical integrity with high porosity to promote cell infiltration and angiogenesis. Likewise, biochemists can produce highly purified, bioactive cytokines in large quantity, suitable for cell culture and in vivo applications. Despite these advances, the availability of stem cells remains a challenge for both scientists and clinicians pursuing regenerative medicine.

By definition, a stem cell is characterized by its ability to self-renew and its ability to differentiate along multiple lineage pathways. Ideally, a stem cell for regenerative medicinal applications should meet the following criteria[2]:

1. Can be found in abundant quantities (millions to billions of cells)
2. Can be harvested by a minimally invasive procedure
3. Can be differentiated along multiple cell lineage pathways in a regulatable and reproducible manner.
4. Can be safely and effectively transplanted to either an autologous or allogeneic host
5. Can be manufactured in accordance with current Good Manufacturing Practice guidelines

This review focuses on human subcutaneous adipose tissue depots as a potential source of adult or somatic stem cells; when cells derived from experimental animal models are discussed, their specie of origin will be identified. With the increased incidence of obesity in the United States and abroad, subcutaneous adipose tissue is abundant and readily accessible.[3] Approximately 400,000 liposuction surgeries are performed in the United States each year.[4] These procedures yield anywhere from 100 mL to >3 L of lipoaspirate tissue.[4] This material is routinely discarded. As discussed below, adipose-derived stem cells are multipotent and hold promise for a range of therapeutic applications. Just as individuals around the world altruistically participate in "blood drives" to donate their circulatory cells for the medical treatment of others, future citizens may undergo liposuction to remove excess adipose tissue in "fat drives."

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From the Stem Cell Biology Laboratory and Clinical Nutrition Research Unit, Pennington Biomedical Research Center (J.M.G.), Louisiana State University System, Baton Rouge; Department of Plastic and Maxillofacial Surgery (A.J.K.), University of Virginia, Charlottesville; and Tulane University Primate Research Center (B.A.B.), Covington, La.

Correspondence to Dr Jeffrey Gimble, Pennington Biomedical Research Center, 6400 Perkins Rd, Baton Rouge 70808. E-mail gimblejm@pbrc.edu

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Nomenclature and the Name of a Rose

In common with many rapidly developing fields, a variety of names have been used to describe the plastic adherent cell population isolated from collagenase digests of adipose tissue. Each of the following terms has its merits: adipose-derived stem/stromal cells (ASCs), adipose-derived adult stem (ADAS) cells, adipose-derived adult stromal cells, adipose-derived stromal cells (ADSCs), adipose stromal cells (ASCs), adipose mesenchymal stem cells (AdMSCs), lipoblast, pericyte, preadipocyte, and processed lipoaspirate (PLA) cells. Nevertheless, the different names lend confusion to the literature. To address this issue, the International Fat Applied Technology Society reached a consensus to adopt the term "adipose-derived stem cells" (ASCs) to identify the isolated, plastic-adherent, multipotent cell population. This review adheres to this nomenclature and advocates its use by others. Recognizing the validity of the term "stem cell" may be questioned; it is accepted that some investigators will use the acronym to mean "adipose-derived stromal cells."

Lessons From Pathology: Why Would We Think Fat Has Any Stem Cells?

Progressive Osseous Heteroplasia

Human pathologies support the concept that adipose tissue contains multipotent progenitor cells. Children with a rare disease known as progressive osseous heteroplasia (POH) present to clinicians as a result of symptoms related to the formation of ectopic bone within their subcutaneous adipose layer of their skin.[5–7] Histological analysis of these lesions demonstrates the presence of osteoblasts and chondrocytes in addition to adipocytes.[5] POH is an autosomal dominant inherited defect associated with mutations in the GNAS1 gene, responsible for the coupling of transmembrane hormone receptors to adenylate cyclase. Epigenetic influences determine how the mutation of the gene is manifested. Imprinting of the gene attributed to a paternal inheritance of an inactive GNAS1 allele causes POH, whereas maternal inheritance causes pseudopseudohypoparathyroidism.[8–12] Thus, an "inborn metabolic error" implies that adipose tissue–derived stem cells are "tripotent," with the capability of adipogenic, chondrogenic, and osteogenic differentiation potential.

Lipomas and Liposarcomas

Various soft tissue tumors lend further weight to the existence of adipose-derived stem cells. Lipomas and liposarcomas are the most common diagnoses of soft tissue tumors presenting in a clinical setting. Often, these tumors occur in subcutaneous or visceral adipose depots and proliferate slowly. The lipid content of benign lipomas and well differentiated liposarcomas is comparable to that seen in normal adipose tissue. This contrasts to myxoid and dedifferentiated liposarcomas, which contain less triglyceride.[13] Using noninvasive nuclear magnetic resonance detection, these distinctions can be used for diagnostic purposes. The quintessential nuclear hormone receptor associated with adipogenesis, peroxisome proliferator-activated receptor (PPAR)γ, can be found in all histological grades of liposarcomas. Ligands for the PPARγ transcription factor include natural (long-chain fatty acids, prostaglandin J2) and synthetic (thiazolidinedione) compounds,[14] and, in vitro, these agents can induce liposarcoma-derived cells to differentiate into adipocytes.[15] Based on these observations, physicians have used oral thiazolidinediones to treat patients with liposarcomas, thereby reducing cell proliferation and increasing expression of adipocyte gene markers.[16,17] Although it remains to be determined whether ligand-induced adipogenesis is an effective chemotherapy for patients with liposarcoma, the work is consistent with the hypothesis that liposarcomas derive from a stem cell progenitor.

Obesity

Obesity presents further evidence supporting the existence of stem cells within adipose depots. Throughout the world, the incidence of overweight and obese individuals has grown at alarming rates. Multiple factors may contribute to this epidemic at the genetic, epigenetic, and behavioral levels. In vivo models of adipogenesis suggest that the mature adipocyte is a terminally differentiated cell, with limited capacity for proliferation and replication.[18–20] Nevertheless, radioactive tracer studies have found that the turnover rate for cells within adipose depots ranges between 6 to 15 months in humans and rodents.[19,20] Presumably, a stem cell population within adipose tissue is responsible for replacing mature adipocytes through the lifetime of the individual. Some scientists have proposed that a homeostatic mechanism or "adipostat" maintains the total adipose tissue volume at a constant level.[21] With rapid weight loss resulting from dieting, exercise, or liposuction, the adipostat operates to return the total adipose tissue volume of an individual back to its initial level.[22] For example, the removal of a fat pad in rats signals the generation of new adipose tissue.[22] This occurs not only through an increase in the volume of preexisting adipocytes but also through the generation of new adipocytes from a progenitor or stem cell pool.

Isolation Procedure and Characterization

Macro- and Micro-considerations

Adipose tissue derives from the mesodermal layer of the embryo and develops both pre- and postnatally.[23,24] Microscopically, the earliest evidence of adipocytes in humans takes place during the second trimester. Likewise, in porcine embryos, monoclonal antibodies have identified preadipocytes between days 50 to 70 of gestation.[25,26] This coincides with the appearance of fibroblastic cells containing an abundant endoplasmic reticulum, a high nuclear/cytoplasm ratio, perinuclear localization of mitochondria, and the presence of lipid vacuoles. With maturation, the adipocytes exhibit multilocular or unilocular lipid accumulations. The microscopic location of the adipogenic progenitor cells remains controversial, as reflected by the multiple names applied to these cells over the years. It remains to be proven whether the origin of the cells correlates with the endothelial, pericyte, or stromal compartments.[25,26] Certainly, preadipocytes and endothelial cells share common surface antigens, consistent with a common origin.[25,26] The adipocyte progenitor could prove to be a "colony-forming-unit fibroblast" (CFU-F)[27]

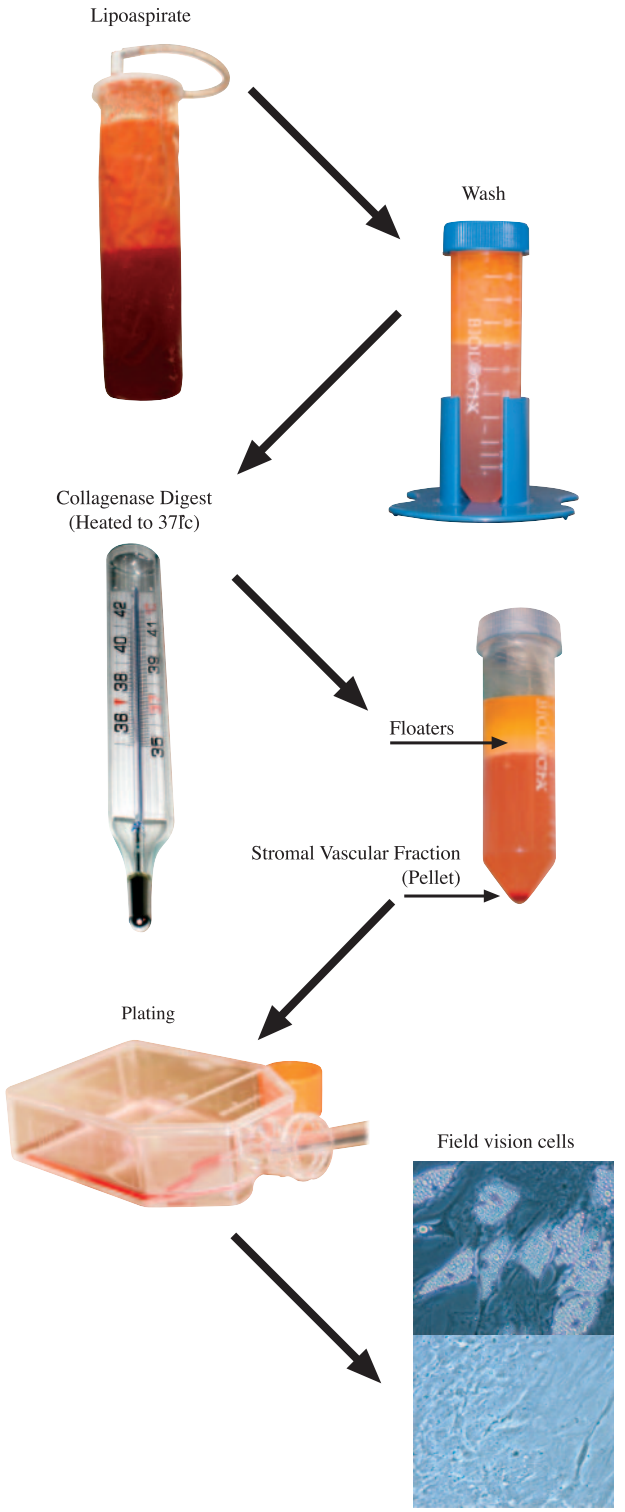
derived from the bone marrow and distributed through the circulation.[28,29] Castro-Malaspina et al[27] originally introduced the concept of a circulating bone marrow–derived fibroblast more than 25 years ago, using the pioneering in vitro CFU-F assay. Recent studies by Crossno et al have used genetically engineered murine models to track transplanted marrow-derived cells.[28] They find that cells similar to the CFU-F can integrate and differentiate into adipocytes within extramedullary adipose depots in response to a thiazolidinedione treatment or a high-fat diet.[28] The contribution of these circulating cells to the overall growth and development of adipose tissue will require further investigation.

Macroscopically, at least 5 different types of adipose tissue exist: bone marrow, brown, mammary, mechanical, and white. Each serves a distinct biological function. In the bone marrow, adipose tissue serves both a passive and active role. It occupies space no longer required for hematopoiesis and serves as an energy reservoir and cytokine source for osteogenic and hematopoietic events. Brown adipose tissue is thermogenic, generating heat through the expression of a unique uncoupling protein that short circuits the mitochondrial pH gradient. Whereas brown adipose tissue is found around the major organs (heart, kidney, aorta, gonads) in the newborn infant, it disappears as humans mature. Mammary adipose tissue provides nutrients and energy during lactation and is regulated, in part, by pregnancy-associated hormones. Mechanical adipose depots, such as the retroorbital and palmar fat pads, provide support to the eye, hand, and other critical structures. Finally, white adipose tissue serves to store energy and provide insulation. There is now a greater appreciation of the role of white and other adipose tissues as an endocrine organ in its own right. Adipose secretion of adiponectin, leptin, resistin, and other adipokines exerts systemic physiological and pathological effects.

Although studies are limited, adipose depot specific differences appear to exist with respect to stem cell content. Whereas multipotent stem cells are abundant within murine white adipose tissue, their numbers and differentiation potential are reduced in brown adipose tissue.[30] In humans, differences in stem cell recovery have been noted between subcutaneous white adipose tissue depots, with the greatest numbers recovered from the arm as compared with the thigh, abdomen, and breast.[30a] Furthermore, it is well established that differences exist with respect to preadipocyte and endothelial cell numbers between subcutaneous and omental white adipose depots in human subjects.[31] It remains to be determined as to which human adipose tissue depot should be harvested for optimal stem cell recovery.

Cell Isolation and Mechanical Devices

The initial methods to isolate cells from adipose tissue were pioneered by Rodbell[32,33] and Rodbell and Jones[34] in the 1960s. They minced rat fat pads, washed extensively to remove contaminating hematopoietic cells, incubated the tissue fragments with collagenase, and centrifuged the digest, thereby separating the floating population of mature adipocytes from the pelleted stromal vascular fraction (SVF) (Figure). The SVF consisted of a heterogeneous cell population, including circulating blood cells, fibroblasts, pericytes,



Processing of lipoaspirate and isolation of adipose-derived stem cells.

and endothelial cells as well as "preadipocytes" or adipocyte progenitors.[32–34] The final isolation step selected for the plastic adherent population within the SVF cells, which enriched for the "preadipocytes." Subsequently, this procedure has been modified for the isolation of cells from human adipose tissue specimens.[35–39] Initially, fragments of human tissue were minced by hand; however, with the development

TABLE 1. Immunophenotype of Passaged Human ASCs

Antigen Category	Surface-Positive Antigens	Surface-Negative Antigens
Adhesion molecules	CD9 (tetraspan), CD29 (β_1 integrin), CD49 days (α_4 integrin), CD54 (ICAM-1), CD105 (endoglin), CD166 (ALCAM)	CD11b (α_b integrin), CD18 (β_2 integrin), CD50 (ICAM-3), CD56 (NCAM), CD62 (E-selectin), CD104 (α_4 integrin)
Receptor molecules	CD44 (hyaluronate), CD71 (transferrin)	CD16 (Fc receptor)
Enzymes	CD10 (common acute lymphocytic leukemia antigen), CD13 (aminopeptidase), CD73 (5′ ecto-nucleotidase), aldehyde dehydrogenase	
Extracellular matrix molecules	CD90 (Thy1); CD146 (Muc18); collagen types I and III; osteopontin; osteonectin	
Cytoskeleton	α-smooth muscle actin, vimentin	
Hematopoietic		CD14, CD31, CD45
Complement cascade	CD55 (decay-accelerating factor), CD59 (protectin)	
Histocompatibility Antigen	HLA-ABC	HLA-DR
Stem cell	CD34, ABCG2	
Stromal	CD29, CD44, CD73, CD90, CD166	

See Refs 50–52, 56, and 57.

of liposuction surgery, this procedure has been simplified. During tumescent liposuction, plastic surgeons infuse the subcutaneous tissues with a saline solution containing anesthetic and/or epinephrine via a cannula and then remove both the liquid and tissue under suction.[40] The procedure generates finely minced tissue fragments, the size of which depends on the dimensions of the cannula. Independent studies have determined that liposuction aspiration alone does not significantly alter the viability of isolated SVF cells.[41–43] Indeed, adherent stromal cells with characteristics of adipocyte progenitors can be found directly within the liposuction aspiration fluid, as well as in SVF derived from the tissue fragment digests.[44] However, when ultrasound-assisted liposuction is performed, the number of cells recovered from tissue digests is reduced, as is their proliferative capacity.[43] The recovery of ASCs can be improved further by manipulating the centrifugation speed.[45] Investigators have achieved optimal cell recovery using a centrifugation speed of 1200*g* based on the subsequent formation of a human-derived adipose tissue depot following implantation in an immunodeficient murine model.[45]

The cell isolation process requires the manipulation of large volumes of lipid-laden cells, presenting potential risks to equipment and personnel. To facilitate the process, several groups have fabricated devices to automate the cell isolation.[4,46] One approach uses a "bag within a bag." The suctioned aspirate flows through a central bag that automatically sieves the tissue while draining away the aspiration fluid. Subsequently, the trapped tissue can be washed and further manipulated.[4] Others have developed a closed, rotating, controlled temperature incubator capable of collagenase digesting and separating up to one liter of tissue at a time.[46] These prototypes may one day lead to commercially available manufactured devices for large scale, automated adipose tissue manipulation and cell isolation suitable for clinical applications.

Immunophenotype

Multiple independent groups have examined the surface immunophenotype of ASCs isolated from human and other species (Table 1).[44,47–51-58] The expression profile changes as a function of time in passage and plastic adherence.[56,57] After 2 or more successive passages in culture, the ASCs express characteristic adhesion and receptor molecules, surface enzymes, extracellular matrix and cytoskeletal proteins, and proteins associated with the stromal cell phenotype. Despite any differences in the isolation and culture procedures, the immunophenotype is relatively consistent between laboratories (Table 1). Indeed, the surface immunophenotype of ASCs resembles that of bone marrow–derived mesenchymal stem or stromal cells (MSCs)[59] and skeletal muscle-derived cells.[60] Direct comparisons between human ASC and MSC immunophenotypes are >90% identical.[52] Consistent with this, the 2 cell populations display similar mitogen-activated protein kinase phosphorylation in response to tumor necrosis factor-α, lipolytic responses to β-adrenergic agents, and adiponectin and leptin secretion following adipogenesis.[61,62] Nevertheless, differences in surface protein expression have been noted between ASCs and MSCs. For example, the glycoprotein CD34 is present on human ASCs early in passage but has not been found on MSCs.[57,59] Not all differences reported in the literature for specific markers are necessarily valid. The Stro-1 antigen, a classic bone marrow MSC-associated surface antigen,[63] was reported as both absent[50] and present[52] on human ASCs. Differences in Stro-1 antibody sources and detection methods (flow cytometry versus immunohistochemistry) could account for this apparent discrepancy in findings.

Identification of the ASC surface immunophenotype has provided a mechanism to enrich or purify the stem cell population directly from the heterogeneous SVF cells.[64–66] Investigators have used immunomagnetic beads or flow cytometry to both positively and negatively select for a subpopulation of cells within the SVF. For example, endothelial progenitors can be removed by negatively selecting for cells expressing CD31 or platelet endothelial cell adhesion molecule-1.[58,64–67] Likewise, positive selection has been performed using CD34 and other antigens. One group working with human adipose tissue has demonstrated that the CD34

TABLE 2. ASC Differentiation Potential

Cell Lineage	Inductive Factors	References
Adipocyte	Dexamethasone, isobutyl methylxanthine, indomethacin, insulin, thiazolidinedione	118, 120
Cardiomyocyte	Transferrin, IL-3, IL-6, VEGF	91, 96
Chondrocyte	Ascorbic acid, bone morphogenetic protein 6, dexamethasone, insulin, transforming growth factor-β	120, 121, 140
Endothelial	Proprietary medium (EGM-2-MV; Cambrex) containing ascorbate, epidermal growth factor, basic fibroblast growth factor, hydrocortisone	65, 98, 99
Myocyte	Dexamethasone, horse serum	120, 136
Neuronal-like	Butylated hydroxyanisole, valproic acid, insulin	127, 128, 141
Osteoblast	Ascorbic acid, bone morphogenetic protein 2, dexamethasone, 1,25 dihydroxy vitamin D_3	120, 132, 142

positive SVF cell population contained the ASCs.[65,66] Species variations may exist; however, studies of murine adipose tissue found that the CD34-negative SVF population was enriched for ASCs based on an in vitro differentiation and in vivo ischemia reperfusion assays.[58,68]

Transcriptome and Proteome

Gene microarrays studies analyzing the transcriptomes of undifferentiated human ASCs and bone marrow–derived MSCs have been reported by independent laboratories.[55,69,70] An analysis of a panel of 28 genes was not significantly different between the 2 cell types.[69] A more comprehensive comparison using Affymetrix gene chips has determined that human ASCs and MSCs share a common transcriptome[71] (R. Izadpanah, B. Bunnell, C. Kriedt, unpublished observation, 2006). The correlation coefficient between the transcriptomes of ASCs and MSCs derived from multiple donors was approximately 50%; this compares to an average correlation coefficient of 71% and 64% between individual donors within the ASC and MSC groups, respectively.[70] The ASCs and MSCs derived from both human and rhesus monkeys express stem cell–associated gene markers, including Oct4, Rex1, and Sox2.[72] Overall, these data suggest that ASC and MSC display distinct but similar expression profiles based on mRNA analyses.

Analyses of the ASC and adipocyte proteome by mass spectrometry and other approaches have documented the identity of >200 proteins in both the undifferentiated and adipose differentiated cells.[73–79] The human ASC proteome shares features in common to that reported for fibroblasts, MSCs, and other lineages.[74,80,81] A direct comparison of ASC and MSC proteomes based on 2D gel electrophoresis has identified 19 individual proteins with >1.5-fold differences (R. Izadpanah, B. Bunnell, C. Kriedt, I. Kheterpal, A. White, unpublished observation, 2006).

Cell Proliferation and Culture

In vitro, ASCs display a cell doubling time of 2 to 4 days, depending on the culture medium and passage number.[56,72] The ASCs maintain their telomere length with progressive passage in culture[72,82]; however, reports differ as to whether the telomerase activity of the ASCs is maintained,[82] decreased with progressive passage,[72] or absent.[55] Interspecies variation does occur; the characteristics of human and rhesus ASCs are similar but not identical.[72] With prolonged passage for >4 months, human ASCs have been observed to undergo malignant transformation.[83] In at least one laboratory, serially passaged ASCs displayed karyotypic abnormalities at a frequency of >30% and, when implanted into immunodeficient mice, formed tumors at a frequency of 50%.[83] These findings indicate that caution should be exercised in the manipulation and culture of the adipose-derived cells. As is discussed below, safety testing for any clinical ASC products will need to include karyotype analysis as well as in vitro and in vivo tumorigenesis assays.

Mechanisms of Potential Utility: How Do ASCs work?

Investigators have postulated a number of nonexclusive mechanisms through which ASCs can be used to repair and regenerate tissues. First, ASCs delivered into an injured or diseased tissue may secrete cytokines and growth factors that stimulate recovery in a paracrine manner. The ASCs would modulate the "stem cell niche" of the host by stimulating the recruitment of endogenous stem cells to the site and promoting their differentiation along the required lineage pathway. In a related manner, ASCs might provide antioxidants chemicals, free radical scavengers, and chaperone/heat shock proteins at an ischemic site. As a result, toxic substances released into the local environment would be removed, thereby promoting recovery of the surviving cells. Exciting studies have suggested that transplanted bone marrow–derived MSCs can deliver new mitochondria to damaged cells, thereby rescuing aerobic metabolism.[84] It may develop that similar studies in ASCs will uncover a comparable ability to contribute mitochondria. A final mechanism is to differentiate ASCs along a desired lineage, as described in the following sections and Table 2.

In all contexts, it is important to consider the potential use of both autologous and allogeneic ASCs. Autologous ASCs offer advantages from regulatory, histocompatibility, and infectious perspectives. As seen with blood cell products, it is often not feasible for an individual patient to provide his or her own therapeutic cell product. Recent studies support further evaluation of allogeneic ASC transplantation. Independent studies from 3 laboratories have determined that passaged human ASCs, as opposed to freshly isolated SVF cells, reduce their expression of surface histocompatibility antigens and no longer stimulate a mixed lymphocyte reaction when cocultured with allogeneic peripheral blood monocytes.[57,85,86] Like bone marrow–derived MSCs,[87] the ASCs actually suppress immunoreactions.[57,85] This indicates that

the ASCs may not elicit a cytotoxic T-cell response in vivo. The hypothesis that transplanted allogeneic ASCs will not elicit a robust immune response and subsequent rejection needs independent and comprehensive testing. If correct, such a finding would have a profound impact on the application of ASCs in regenerative medicine. The ability to transplant allogeneic ASCs will reduce the cost of cell therapies. Quality assurance and control steps can be streamlined once multiple use, as opposed to single donor/recipient, product approval takes place and ASCs are manufactured in large volumes. Likewise, the availability of off-the-shelf allogeneic ASCs will allow physicians and surgeons to use them directly at the point of care, ie, the emergency room, rather than limiting their use to elective procedures.

Functional Tissue Engineering Principles

Regenerative medicine intends to differentiate ASCs and other stem cells along specific lineage pathways to effect repair of damaged or failing organs. To achieve this goal, leaders in the musculoskeletal field have proposed the "functional tissue engineering" guidelines.[1] The following objectives have merit, regardless of the tissue of interest, and should be used to evaluate each potential ASC application whenever possible.

1. Define functional success of the engineered tissue rigorously and before implementation.
2. Develop a thorough understanding of the biomechanical properties of the tissue in vivo and the intrinsic properties of native tissues.
3. Develop design criteria that establish the biomechanical and metabolic requirements of the tissue to be replaced.
4. Design and characterize the biomechanical and biophysical properties of the biomaterial scaffolds that will be used for tissue engineering.
5. Develop a thorough understanding of the biophysical microenvironment of the cells within engineered constructs.
6. Employ biophysical stimuli to control cell differentiation and tissue metabolism in vitro and in vivo.

Mechanisms of Potential Utility: Lineage-Specific Differentiation Potential

Cardiac Disease and the Potential of ASC Cardioplasty

Important experimental findings in recent years suggest considerable therapeutic potential for cellular replacement in the context of acute myocardial infarction (MI) and chronic, progressive cardiac disease (eg, left ventricular remodeling and heart failure). Cardiovascular disease still ranks as the no. 1 cause of death in the United States, responsible for nearly 38% of the more than 2.4 million Americans who die each year. Medical expenses and disability resulting from cardiovascular disease were estimated to cost Americans approximately $370 billion in 2004.[87a] The development of novel, effective cell-based therapies could thus have a major societal impact by improving patient outcome after MI.

Cellular cardioplasty is a rapidly progressing field and a large body of work now exists pertaining to its therapeutic potential. A PubMed search of "cardiac disease" and "stem cells" from 1996 to present returns over 1000 hits; adding the term "clinical trials" returns over 60 hits. Although the majority of cellular cardioplasty research has involved the use of myoblasts and/or marrow-derived cells, ASCs represent a viable alternative option.

Although the in vitro plasticity of stromal cells isolated from human subcutaneous adipose tissue is now well established,[88] only a handful of reports exist relating to their differentiation into the cardiomyocyte lineage. The first of these reports communicates the differentiation of adipose-derived cells from rabbits using 5-azacytidine and delineates the cardiomyocyte lineage based on cell morphology, spontaneous contraction, and positive immunostaining for myosin heavy chain, α-actinin, and troponin-I.[89] Gaustad et al report the cardiac differentiation of human ASCs from a 38-year-old woman by reversibly permeabilizing the cells and exposing them to rat cardiomyocyte extract.[90] They also delineate the cardiomyocyte lineage based on morphology (binucleated, striated cells), spontaneous beating, and the expression of sarcomeric α-actinin, desmin, cardiac troponin I, and connexin 43. Planat-Benard et al report the spontaneous differentiation of murine ASCs into cardiomyocytes without the use of 5-azacytidine.[91] Both ventricle- and atrial-like cells were described based on extensive characterization that included morphological observations, ultrastructural analysis, expression of cardiac markers, electrophysiological studies, and functional studies that evaluated the response of the cells to adrenergic and cholinergic agonists. None of the studies summarized above evaluated the described cells within an in vivo setting.

An equally limited number of studies have explored the use, behavior and effect of ASCs in the in vivo setting of myocardial damage. Animal models of MI can be quite complicated, and multiple factors must be considered when evaluating and comparing such studies. Some of the variables to consider include:

1. The species and strain used (eg, mouse, rat, dog, pig; immunocompetent versus immunocompromised)
2. The specific model (eg, acute MI or chronic heart failure; cryoinjury or ischemic occlusion; reperfusion after ischemia, or not)
3. Immunological aspects of the "donor" cells (eg, autologous/syngeneic, allogeneic, xenogeneic)
4. Method, site, and timing of cell delivery (eg, direct intramuscular injection, peripheral IV, coronary vessels, LV injection)
5. Imaging and quantitative methods (eg, MRI, echocardiography, nuclear imaging, histology)

Each one of these elements has the potential to significantly impact the findings and conclusions of a given study, not to mention the variables associated with cell isolation and preparation.

One of the first published reports of ASCs within the myocardial milieu involved the use of transgenic murine ASCs within a murine cryoinjury model of myocardial damage.[92] Initial in vitro differentiation studies using 5-azacytidine demonstrated the upregulation of various cardiac-specific markers in treated cells. For the in vivo studies, uncultured syngeneic murine ASCs were injected into the left ventricular chamber immediately after cryoinjury

and hearts were evaluated by histology up to 14 days postsurgery. Although no structural or functional analysis was performed, immunohistochemical staining revealed myocardial engraftment of ASCs with concomitant expression of myosin heavy chain, troponin I, and the transcription factor Nkx2.5.[92]

Katz et al demonstrated the engraftment of culture-expanded human ASCs into infarcted myocardium using an immunocompromised murine model of acute reperfused MI.[93] Cells were delivered by direct injection 20 minutes after reperfusion and subsequently identified postmortem using fluorescent labeling, 5-bromodeoxyuridine, and intracellular superparamagnetic iron oxide (SPIO) particles. MRI was used to noninvasively track cell position as well as to quantify cardiac structure and function. Although engrafted ASCs did not assume a morphology or demonstrate expression of proteins consistent with a cardiomyocyte phenotype, trends toward enhanced cardiac structure and function were detected in cell-treated mice compared with historical controls.

A recent article from Japan reports the efficient differentiation of $CD29^+$ murine ASCs from brown adipose tissue (BAT) into cardiomyocyte-like cells based on morphology, molecular and protein expression profile, and electrophysiological parameters.[94] These cells were then tested in vivo using transgenic green fluorescent protein rat ASCs implanted into rat hearts after acute MI by coronary ligation. Echocardiography was used to assess cardiac function, revealing enhanced function and decreased remodeling compared with saline controls. Immunohistochemical analysis demonstrated that implanted cells expressed markers consistent with endothelial cells, smooth muscle cells, and cardiomyocytes. Interestingly, $CD29^+$ cells derived from white adipose tissue did not demonstrate these same properties, making translation of these findings to the clinical setting somewhat challenging.

In addition, Miyahara et al report the novel and intriguing use of ASCs grown as monolayer sheets for myocardial repair.[95] Rat ASCs were isolated, flow characterized, and grown as intact monolayer sheets using temperature-responsive culture dishes. The ASC sheets were shown to secrete significantly more angiogenic and antiapoptotic factors than sheets composed of dermal fibroblasts. Placement of the ASC sheets onto scarred myocardium 4 weeks after coronary ligation in rats resulted in decreased scarring and enhanced cardiac structure and function compared with untreated controls and dermal fibroblast treated animals. Histological analysis demonstrated that the engrafted ASC sheets grew to form a thickened layer over the infarcted muscle that included newly formed vessels and some cardiomyocytes.

Finally, Song et al have identified vascular endothelial growth factor (VEGF) as a critical factor in cardiomyogenesis in human ASCs.[96] They observed spontaneous cardiomyocyte differentiation of freshly isolated human ASCs over a 12-day period based on the expression of Nkx2-.5, GATA-4, cardiac troponin T, and myosin chain 2 v.[96] These processes associated with high levels of VEGF expression by the human ASCs and could be inhibited by the presence of an anti-VEGF antibody, suggesting a possible paracrine mechanism of action.[96]

Although limited in volume, the existing literature does suggest that ASCs are able to engraft and survive within an infarcted myocardial milieu, acquire phenotypic markers consistent with cardiomyocyte and vascular-related lineages, and positively impact structural and functional end points. Despite these encouraging results, however, a great deal remains to be learned about cell-based therapies for myocardial damage.

Endothelial

The preadipocytes within adipose tissue depots and endothelial cells exhibit close relationships in vivo.[97] Following isolation, cells within the heterogeneous SVF express markers consistent with an endothelial phenotype including CD31, CD144 (VE-cadherin), and von Willebrand factor.[65,68,98–100] These should be distinguished from ASCs because they have demonstrated lineage commitment. Williams and colleagues pioneered the isolation of such differentiated endothelial cells from human adipose tissue.[48,101] By screening for collagenase enzymes with specific characteristics, they recovered cells expressing von Willebrand factor.[102] They were able to seed these endothelial cells onto synthetic vascular grafts and improve the patency of these grafts following surgery.[47,48,101] Alternatively, they successfully transplanted the cells into the corpus cavernosum of rats, suggesting that they could be used in the treatment of erectile dysfunction.[103]

Others have documented the ability of SVF cells and culture expanded ASCs to undergo endothelial differentiation in vitro.[65,66,68,98–100,104] Planat-Benard et al[98] observed that $CD13^+CD34^+$ SVF cells cultured in Matrigel expressed CD31 and von Willebrand factor. Moreover, the cells formed branching networks, consistent with the formation of vascular structures. Miranville et al[65] reported similar findings using a population of $CD31^-CD34^+$ SVF cells. The addition of VEGF enhanced their differentiation, based on expression of CD31 and the development of vascular structures in Matrigel. In related work, Cao et al[68] demonstrated that a population of $CD31^-CD34^-CD106^-$, fetal liver kinase-1^+ (flk-1) adipose-derived cells could express endothelial markers in response to VEGF. Likewise, Martinez-Estrada et al[104] found that flk-1^+ cells isolated from cultured ASCs displayed an endothelial phenotype in the presence of VEGF. In vivo studies further support the endothelial differentiation potential of SVF cells. In a rat ischemic hindlimb model, the introduction of the $CD31^-$ population improves angiogenesis and subsequent recovery of vascular supply.[65,68,98,99,105] This finding has been confirmed by at least 5 independent groups and is reproduced both when the cells are delivered intravenously[65,68,99] or by intramuscular injection in the direct vicinity of the ischemic injury.[98,105] Although the ASCs can integrate as fully functional and differentiated endothelial cells in vivo, they may contribute additionally through paracrine pathways. The adipose-derived cells secrete angiogenic cytokines such as VEGF and hepatocyte growth factor (HGF), and these are postulated to contribute to the ASC angiogenic properties.[99,105] The levels of VEGF and/or HGF secreted by ASCs can be induced by exposure of the cells to hypoxia,[99] growth and differentiation factor 5,[106] tumor necrosis factor-α,[107] basic fibroblast growth factor, epidermal growth factor, and

ascorbate.[108] In vivo studies using the murine 3T3-F442A preadipocyte model suggest that a reciprocal relationship may exist between angiogenesis and adipogenesis.[109] This involves VEGF, because the introduction of VEGF receptor antibodies interferes with the formation of new adipose depots in vivo, which is consistent with a paracrine pathway between endothelial and preadipocyte cells.[109] Further support for a paracrine relationship between these lineages comes from the observation of increased proliferative rates noted in 2D and 3D cocultures of preadipocytes and endothelial cells.[64,110]

Smooth Muscle

In vitro, human ASCs can express α-smooth muscle actin, calponin, and SM22, consistent with a smooth muscle phenotype.[111,112] The addition of transforming growth factor-β and sphingosylphosphorylcholine induces human ASC expression of smooth muscle–associated markers.[111,112] Overexpression studies in a preadipocyte murine model indicate that the aortic carboxypeptidase-like protein may underlie the mechanism of ASC smooth muscle differentiation.[113,114] In addition, mechanical stimuli modulate the smooth muscle phenotype; cyclic uniaxial strain reduced human ASC expression of α-smooth muscle actin and calponin.[115] In vivo, human ASCs persist for up to 12 weeks and display the morphology of smooth muscle cells when injected into the urinary tract of immunodeficient mice.[116] Technological developments allowing for the controlled generation and manipulation of ASC sheets may provide methods to deliver smooth muscle–differentiated ASCs for genitourinary and cardiovascular regenerative procedures.[95,117]

Evidence for Additional Lineage Differentiation

There is a growing body of experimental evidence from both in vitro and in vivo studies demonstrating the multipotentiality of ASCs from adipose tissue isolated from humans and other species. These include the adipocyte,[52,118–120] chondrocyte,[52,120–122] hematopoietic supporting,[108,123] hepatocyte,[124,125,126] neuronal-like,[52,53,127–130] osteoblast,[52,120,131–133] pancreatic,[134] and skeletal myocyte[52,120,135,136] pathways. Because these lineage pathways fall outside of the scope of the current review, we refer the reader to in-depth reviews for further information on these subjects.[88,137–139]

Moving Into the Future: The Challenges

Many important scientific and medical questions remain. These include the development of large scale manufacturing methods with appropriate quality assurance and quality control to generate cells in compliance with current Good Manufacturing Practices. Preferably, these steps will be performed in a closed, sterile container that minimizes risk for contamination of the tissue while protecting the personnel from exposure to bloodborne pathogens. Cell culture in stirred or rotating flasks, perfused hollow fiber bioreactors, or Teflon bags may be required. It will be valuable to develop a serum protein free culture medium to avoid issues related to bovine spongiform encephalopathy or other xenogeneic infections. Finally, it will be important to determine how to store the ASCs, what kind of containers to store them in, how to label those containers, how to ship them to the point of care, and how long their shelf life will be.

Recent studies have demonstrated that human ASCs, after prolonged culture in vitro, are capable of forming tumors in immunodeficient mice.[83] Consequently, long-term experiments examining the safety of ASC transplantation in appropriate animal models will be required. Such studies should be designed in consultation with regulatory agencies to insure that all preclinical questions are addressed before advancing to phase I studies in patients. If and when ASCs are approved for clinical use, physicians and health care professionals will need to be educated regarding their unique properties and correct application.

Hope and Hype

Mass media have covered the application of ASCs and other stem cells for the treatment of a wide range of medical conditions. Not surprisingly, US citizens have high expectations for the promise that these therapies will offer in the future. With time and resources, it is likely that some of these goals will be realized. Nevertheless, it is important for the scientific and medical communities to balance the hope from the hype. This balance should always be addressed whenever communicating new data or studies to the media. Time will only tell whether, someday, healthy individuals will voluntarily undergo liposuction to donate fat in the same way they now donate blood.

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