REVIEW ARTICLE

Adipose – Derived Stem Cells for Future Regenerative System Medicine

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Abstract

BACKGROUND: The potential use of stem cell-based therapies for repair and regeneration of various tissues and organs offers a paradigm shift that may provide alternative therapeutic solutions for a number of diseases. Despite these advances, the availability of stem cells remains a challenge for both scientists and clinicians in pursuing regenerative medicine.

CONTENT: Subcutaneous human adipose tissue is an abundant and accessible cell source for applications in tissue engineering and regenerative medicine. Routinely, the adipose tissue is digested with collagenase or related lytic enzymes to release a heterogeneous population for stromal vascular fraction (SVF) cells. The SVF cells can be used directly or can be cultured in plastic ware for selection and expansion of an adherent population known as adipose-derived stromal/stem cells (ASCs). Their potential in the ability to differentiate into adipogenic, osteogenic, chondrogenic and other mesenchymal lineages, as well in their other clinically useful properties, includes stimulation of angiogenesis and suppression of inflammation.

Abstrak

LATAR BELAKANG: Potensi penggunaan terapi berbasis *stem cell* untuk memperbaiki dan regenerasi berbagai jaringan dan organ memberikan paradigma baru dalam penyediaan alternatif terapi berbagai jenis penyakit. Akan tetapi di balik semua itu, ketersediaan *stem cell* tetap menjadi tantangan bagi ilmuwan maupun klinisi di dalam *regenerative medicine*.

ISI: Jaringan adiposa subkutan meruapakn sumber sel yang berlimpah untuk diaplikasikan dalam *tissue engineering* dan *regenerative medicine*. Jaringan adipose ditambahkan *collagenase* atau *lytic enzymes* untuk melepaskan populasi *stromal vascular fraction* (SVF) *cells*. *SVF cells* dapat digunakan langsung atau dapat dikultur untuk selanjutnya dilakukan seleksi dan ekspansi dari *adherent population* yang diketahui sebagai *adipose-derived stromal/stem cells* (ASCs). ASC memiliki potensi untuk berdiferensiasi menjadi *lineage adipogenic, osteogenic, chondrogenic* dan *lineage mesenchymal* lainnya, serta penggunaannya secara klinis, meliputi stimulasi angiogenesis dan menekan inflamasi.

SUMMARY: Adipose tissue is now recognized as an accessible, abundant and reliable site for the isolation of adult stem cells suitable for the application of tissue engineering and regenerative medicine applications. The past decade has witnessed an explosion of preclinical data relating to the isolation, characterization, cryopreservation, differentiation, and transplantation of freshly isolated stromal vascular fraction cells and adherent, culture-expanded, adipose-derived stromal/stem cells *in vitro* and in animal models.

KEYWORDS: Adipose tissue, adult stem cells, regenerative medicine, mesenchymal stem cells

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RINGKASAN: Jaringan adiposa dikenal sebagai jaringan yang mudah diakses, berlimpah dan terpercaya untuk isolasi *adult stem cell* yang cocok untuk aplikasi *tissue engineering* dan *regenerative medicine*. Studi terdahulu telah menunjukkan data preklinis terkait dengan isolasi, karakterisasi, *cryopreservation*, diferensiasi dan transplantasi *freshly isolated stromal vascular fraction cells* dan *adherent*, *culture-expanded*, *adipose-derived stromal/stem cells* baik *in vitro* maupun pada *animal models*.

KEYWORDS: *Adipose tissue*, *adult stem cells*, *regenerative medicine*, *mesenchymal stem cells*

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Introduction

The therapeutic potential of multilineage stem cells is enormous for their possible applications in tissue engineering and gene therapy (1). Essentially, there are only two types of stem cells: the embryonic stem cell (ES cell) and adult stem cell. The ES cell, as its name implies, is derived from embryo, or more specifically, from the blastocyst's inner cell mass. In contrast, the adult stem cell is derived from postnatal tissues and can include fetal-derived stem cells and umbilical cord blood stem cells. Over the last 10 years, giant strides have been made worldwide in the field of adult stem cell. In 2002, researchers at UCLA published a manuscript in Molecular Biology of the Cell describing a novel adult stem cell population isolated from adipose tissue – the adipose-derived stem cell (ASC). Since that time, the ASC has become to be one of the most popular adult stem cell populations currently being used in the stem cell field (2).

With the increased incidence of obesity worldwide, subcutaneous adipose tissue is abundant and readily accessible. Approximately 400,000 liposuction surgeries are performed in the United States each year. One procedure of the surgery may yield from 100 mL to > 3 L of lipoaspirate tissue; this material is routinely discarded (3,4).

Similar to other rapidly developing fields, a variety of names have been used to refer to the plastic adherent cell populations isolated from collagenase digests of adipose tissue: 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. To prevent confusion that may arise from the several different names, the International Fat Applied Technology Society has reached to a globally-agreed consensus to adopt the term "adipose-derived stem cells (ASCs)" to refer to the isolated, plastic-adherent, multipotent cell population (5).

The possible feature of ASC being pluripotent cells may obviously lead to a turning point in the stem cell field. Today, the strongly proposed uses of ASCs in tissue repair/regeneration are quite impressive. Hot areas of research in this field include ischemia revascularization, cardiovascular tissue regeneration, bone/cartilage repair, and urinary tract reconstruction (2). Liver injury repair may also be possible by transplantation of rat ASCs, decreasing key liver enzyme levels and increasing serum albumin (6). Even diabetes may be a target for ASC therapy with murine ASCs that can reduce hyperglycemia in diabetic mice (7). Most recently, researchers have begun to explore the potential uses of 'reprogrammed" ASCs as iPS (induced pluripotent stem) cells and suggested that the ASC may be easier to reprogram than the fibroblast (8).

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 human and other species. These include the adipocyte, the chondrocyte, hematopoietic supporting,

hepatocyte, neuronal-like, osteoblast, pancreatic, and skeletal myocyte pathways (5,9-30). There is no other human tissue as expendable as the adipose tissue, making it relatively easy to isolate adequate number of ASCs for possible human therapies. With this fact, together with the early clinical uses of ASCs with no adverse effects being reported, it seems only a matter of time before more and more clinical applications of ASCs are reported (2).

Adipose Tissue, Its Cellular Components

Historically, adipose tissue (AT) has been thought to play a passive metabolic role, acting solely as an energy storage reservoir (31). This view has changed, and now adipose tissue is considered an important endocrine organ that provides plastic properties (32,33). Recently, adipose tissue has been also reported as an important reservoir of stem cells with possible practical uses in medicine (34).

Three functionally different types of adipose tissues are classically described in mammals, namely Brown Adipose Tissue (BAT), White Adipose Tissue (WAT) and Bone Marrow Adipose Tissue (BMAT) (35-37). Brown and white adipocytes display both lipolytic and lipogenic activities, but the main role of white adipocytes is to store and mobilize energy as triglycerides, while brown adipocytes are specialized in energy dissipation as heat. Bone marrow adipocytes can contribute to haematopoiesis and osteoblastogenesis by acting as metabolic stores but also via their paracrine activities (38).

Excessive AT development is thought to be the result of both adipocyte hypertrophy and apparent hyperplasia (39-43). As adipocytes are terminally differentiated cells, and as such are considered incapable of division (44,45), the apparent increase in adipocyte number is thought to originate from adipogenesis (the proliferation/differentiation of adipocyte progenitor cells named preadipocytes). Thus, the expansion of adipose mass requires the presence of adipocyte precursor cells located in the stroma vascular fraction of AT and the presence of which could also contribute to AT normal cell turnover during adulthood as recently suggested (46). The search for the origin of adipocyte progenitors revealed that AT hosts a population of multipotent progenitors called adipose-derived stromal cells (ASCs) (5,7). These progenitors can give rise to osteoblasts, chondrocytes and adipocytes (5). They also participate in and/or support angiogenesis or vascular repair in ischemic limbs (47-49) and have immuno-modulatory properties both *in vitro* and *in vivo* (50-51).

AT is composed mainly of fat cells organized into lobules (52). It is a highly complex tissue consisting of mature adipocytes, which constitutes more than 90% of the tissue volume, and a SVF, which includes preadipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, resident monocytes/macrophages, lymphocytes, and ASCs (53,54). ASCs harvested from superficial abdominal regions are significantly more resistant to apoptosis than ASC harvested from the upper arm, medial thigh, trochanteric part, and superficial deep abdominal depots (55). The density of stem cell reserves varies within adipose tissue and is a function of location, type and species (e.g. human vs murine). In white adipose tissue, for example, ASC yields are greater in subcutaneous depots than in visceral fat, with the highest concentrations being in arm adipose tissue depots and the greatest plasticity in ASCs isolated from inguinal adipose tissue depots (56).

Isolated from the SVF of AT, ASCs bear a strong resemblance to bone marrow stem cells (BMSC) as demonstrated by their expression of common cell surface markers, their similar gene expression profiles, and their similar differentiation potentials (5,57,58). Unlike BMSC, however, ASC can be obtained in large quantities at low risks (59). In addition to being more abundant and easily accessible, the adipose tissue yields far more stem cells than bone marrow on a per gram basis (5,000 vs 100-1,000) (60). Therefore, it is reasonable to expect that ASC will become the preferred choice of adult stem cells for future clinical applications.

ASCs and Stromal Vascular Fraction

Subcutaneous fat is an abundant and accessible source of both uncultured/heterogeneous SVF cells and cultured/relatively homogeneous ASCs (61). The molecular phenotype of ASC has only recently been clarified (62), following many years of referring to any cultured SVF cells as ASC. Freshly isolated SVF contains a mixture of cells, which not only includes ASC but also contains endothelial cells, smooth muscle cells, pericytes, fibroblasts, and circulating cell types such as leukocytes, hematopoietic stem cells, or endothelial progenitor cells (63,64). Through collagenase digestion, a heterogeneous cell mixture containing all cell types, except adipocytes, can be extracted from adipose tissue (or liposuction

aspirates) as a cell pellet. Adipocytes are disrupted into oil during the process and discarded as floating tissue and oil after centrifugation. The sedimented cell fraction is called the stromal vascular fraction (SVF) and is basically stromal cells along with vascular endothelial and mural cells (52). Study identified freshly isolated ASCs as $CD31^-CD34^+CD45^-CD90^+CD105^-CD146^-$cells, but they become $CD105^+$ when plated (65). Nucleate cells contained in the SVF obtained from lipoaspirates are composed of 37% leukocytes ($CD45^+$), 35% ASCs ($CD31^-CD34^+CD45^-$), 15% endothelial cells ($CD31^+CD34^+CD45^-$) and other cells ($CD31^-CD34^-CD45^-$), although the percentage of blood-derived cells strongly depends on individual hemorrhage volume (66). ASCs can be extracted from both the floating fatty portion and fluid portion of liposuction aspirates; however, the fluid portion contains much fewer adipose-derived cells and may more blood-derived cells. ASCs can be used clinically without cell expansion if harvested from a large volume of lipoaspirates because a sufficient number of cells can be obtained; 0.1-1 billion nucleate cells can be obtained from 200 mL of aspirated fat tissue and at least 10% of these are ASCs. The use of freshly isolated cells likely leads to higher safety and efficacy in treatments compared with cells expanded by culture (52).

The fact that stem cells yields are greater from AT than from other stem cell reservoirs is another significant factor in their suitability for use in regenerative medicine. Routinely, $1x10^7$ adipose stromal/stem cells have been isolated from 300 mL of lipoaspirate, with greater than 95% purity (67). In other words, the average frequency of ASC in processed lipoaspirate is 2% of nucleated cells, and the yield of ASCs is approximately 5,000 fibroblast colony-forming units (CFU-F) per gram of adipose tissue, compared with estimates of approximately 100-1,000 CFU-F per milliliter of bone marrow (60). Several groups have demonstrated that mesenchymal cells within the SVF of subcutaneous adipose tissue display multilineage developmental plasticity. These cells have alternatively been referred to as processed lipoaspirate cells (PLA), adipose-derived stem cells, and adipose-derived mesenchymal progenitor cells. It is also likely that cells previously considered preadipocytes are essentially the same population (9,68).

Mesenchymal stem cells seem to be an ideal population of stem cells for practical regenerative medicine, because they are not subjected to the same restrictions. In particular, large number of ASCs can be easily harvested from adipose tissue. Furthermore, recent basic research and preclinical studies have revealed that the use of ASCs in regenerative medicine is not limited to mesodermal tissue but extends to both ectodermal and endodermal tissues and organs although ASCs originate from mesodermal lineages (69). The proliferation capacity of ASCs seems to be greater than that of bone marrow-derived mesenchymal stem cells. Previous reports have shown that the doubling times of ASC during the logarithmic phase of growth range from 40 to 120 hours (14,70,71) are influenced by donor age, type (white or brown adipose tissue), the harvesting procedure, culture conditions, plating density and media formulations (63,72). The younger the donor, the greater the proliferation and cell adhesion of the ASCs, while cells gradually lose their proliferative capacity with passaging (71). Based on β-galactosidase activity, senescence in ASCs is similar to that in bone marrow-derived MSCs (70).

The proliferation of ASCs can be stimulated by a single growth factor such as fibroblast growth factor (FGF)-2, endothelial growth factor (EGF), insulin-like growth factor (IGF)-1 or TNF-α (72,73). FGF-2, in particular, is an effective growth-stimulating factor and is required for the long-term propagation and self-renewal of ASCs via the extracellular signal-related kinase (ERK) 1/2 signaling pathway (74). The proliferation of ASCs can also be stimulated by platelet-derived growth factor (PDGF) via Jun amino-terminal kinase (JNK) activation (75) and by oncostatin M via activation of the microtubule-associated protein kinase/ERK and the JAK3/STAT1 pathways (76). ASCs proliferation is also reported to be enhanced by multiple growth factors, which can include any of the single factors mentioned above supplemented by thrombin-activated platelet-rich plasma (77), human platelet lysate (78) and human thrombin (79).

As far as the differentiation into cells of the mesodermal lineages and regeneration of mesodermal tissues are concerned, ASCs can differentiate into adipogenic (80-82), osteogenic (83), chondrogenic (83-85), myogenic (30), cardiomyogenic (86-87), angiogenic (48,88), tenogenic (89), and periodontogenic lineages (90), and tissue regeneration studies with suitable scaffolds and growth factors in appropriate external environment have been carried out (91,92). It has been shown that ASCs can differentiate into endoderm lineage cells. Several reports have shown that ASCs have the potential to differentiate into hepatocytes as indicated by the presence of hepatocyte growth factor (HGF) and FGF-1 (93,94). In theory, ASCs could be used to reduce liver inflammation and treat liver fibrosis by differentiating directly into hepatocytes or by secreting factors such as angiogenic, anti-apoptotic, anti-inflammatory, and anti-fibrotic factors. In addition to hepatic differentiation, the exposure of ASCs to nicotinamide, activin-A, exendin-4, HGF, and pentagastrin resuted in the production of pancreatic-

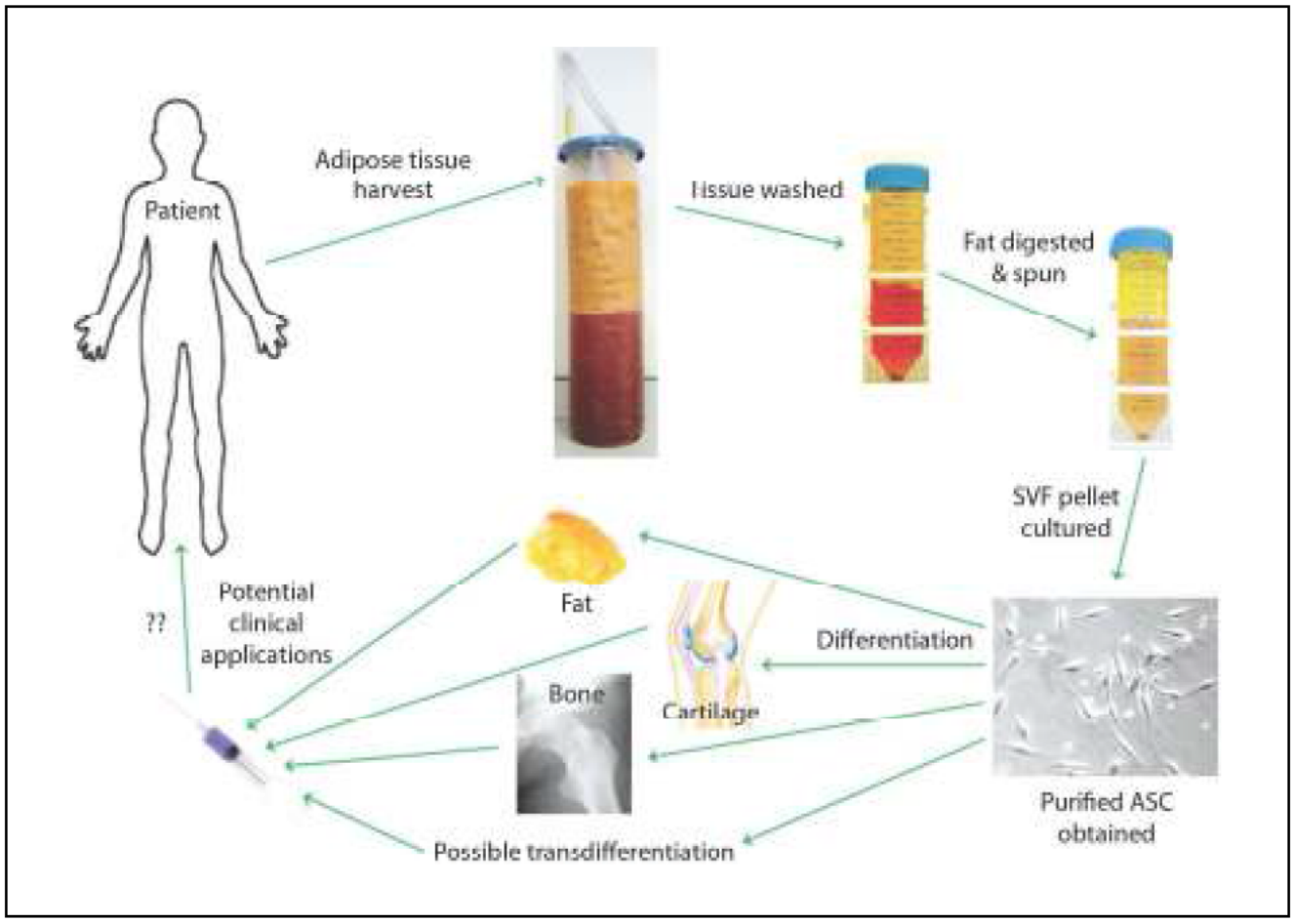

Figure 1. The Process of ASC Clinical Usage (adapted with permission from John Wiley and Sons 2012)

like ASCs capable of insulin, glucagon and somatostatin secretion (28,95). The recent International Federation of Adipose Therapeutics and Science meeting in Dallas, Texas (October 22-24, 2010) demonstrated some of the many novel uses of lipoaspirate, SVF and ASC that are currently being investigated to solve clinical problems (96).

Cytokine Profile and Immunogenicity of ASCs

Adipose tissue serves as source of adipokines and cytokines with both local and systemic actions in health and disease (97). It has been shown that the beneficial impact on different organ/tissues within the human body may be due to soluble factors produced by ASCs rather than their differentiation capability toward different mature lineages (98). Analysis of the soluble factors released from human ASCs have revealed that cultured ASCs, at relatively early passages, secrete hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), insulin-like growth factor (IGF)-1, basic fibroblast growth factor (bFGF), granulocyte-macrophage colony stimulating factor, TNF-α, interleukin (IL)-6, IL-7, IL-8 and IL-11, adiponectin, angiotensin, cathepsin D, pentraxin, pregnancy zone protein, retinol-binding protein, and CXCL12 (48,97,99). Thus, it may be that when ASCs are transplanted into inflammatory or ischemic regions, they actively secrete these growth factors, thereby significantly promoting wound healing and tissue repair (69). Thus, ASCs display cytokine secretory properties similar to those reported for bone marrow-derived mesenchymal stem cells (MSCs) (97).

Multiple independent groups have examined the surface immunophenotype of ASCs isolated from human and other species (9,10,65,100-107). The expression profile changes as a function of time in passage and plastic adherence (63,106). 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. Indeed, the surface immunophenotype of ASCs resembles that of bone marrow-derived mesenchymal stem or stromal cells (MSCs) (108) and skeletal muscle-derived cells (109). Direct comparison between human ASC and MSC immunophenotypes are > 90% identical (9). 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 (110-116). The human ASC proteome shares features in common to that reported for fibroblasts, MSCs, and other lineages (111,117,118).

Freshly isolated SVF cells are a heterogeneous cell population that includes putative ASCs ($CD31^-$, $CD34^+/$, $CD45^-$, $CD90^+$, $CD105^-$, and $CD146^-$), endothelial (progenitor) cells ($CD31^+$, $CD34^+$, $CD45^-$, $CD90^+$, CD105, and $CD146^+$), vascular smooth muscle cells or pericytes ($CD31^-$, CD $34^+/^-$, $CD45^-$, $CD90^+$, $CD105^-$, and $CD146^+$), and hematopoietic cells (CD45+) in uncultured conditions (119). Additionally, compared with ASCs from later passages, freshly isolated SVF cells and early passages ASCs express higher levels of CD117 (c-kit), human leukocyte antigen-DR (HLA-DR), and stem cell-associated markers such as CD34, along with lower levels of stromal cell markers such as CD13, CD29 (β1 integrin), CD44, CD63, CD73, CD90, CD105, and CD116 (63,65,68,103,119-132). While the consequences of the decrease in CD34 expression in later passage ASCs are not clear, there is al least one study demonstrating that CD34 expression can be maintained during 20 weeks of culture (65). As indicated, ASCs share many cell surface markers with pericytes and bone marrow-MSCs. Except for those mentioned above, the pericyte markers expressed by ASCs include smooth muscle β-actin, platelet-derived growth factor (PDGF) receptor-β, and neuro-glial proteoglycan 2 (121), while the markers shared by ASCs and MSCs include CD13, CD29, CD44, CD58 and CD166. Finally, Puissant *et al.* (50) have reported on the lack of HLA-DR expression and the immunosuppressive properties of human ASCs. Based on such findings, Fang et al published preliminary data showing that severe steroid-refractory acute graft-versus-host disease (GVHD) could be treated with human ASCs from HLA-mismatched donors (133).

Clinical Translation of ASCs

Adipose tissue is a source of freshly isolated, heterogeneous stromal vascular fraction cells and culture-expanded, adherent, and relatively homogeneous adipose stromal/stem cells. Both population display regenerative capcity in soft and hard tissue repair, ischemic insults and autoimmune diseases. While their major mechanism of action has been attributed to both direct lineage differentiation and/or paracrine factor release, current evidence favors a paracrine mechanism. Over 40 clinical trials using adipose-derived cells conducted in 15 countries have been registered with the NIH, the majority of which are Phase I or Phase I/II safety studies. Explorations into the regenerative potential of adipose-derived cells occur throughout the world (134). In Asia and Europe, regulatory approval has been granted for closed mechanical devices for adipose tissue processing and SVF cell isolation using collagenase digestion (135). The avaibility of these machines in operating rooms has facilitated the point-of-care delivery of SVF cells for investigators and clinicians in Asia and Europe. Clinical applications using adipose-derived cells are underway throughout Asia, Europe and North and South America. Some of these can be found on the NIH's website (136), where 55 studies are listed under the search term 'adipose stem cell' (as of 22 October 2011). Of these, 44 studies actually employ adipose-derived cells or tissues for regenerative applications (134).

There remains some dispute over the criteria defining an SVF cell or an ASC. While there is a general consensus that the SVF cells are a heterogeneous population, no specific ranges for each subpopulation have been agreed upon formally. The International Society for Stem Cell Therapy (ISCT) has provided guidelines for the definition of mesenchymal stromal cells (MSCs) based on their plastic adherent properties, immunophenotype ($CD73^+$ $CD90^+$ $CD105^+$ $CD11b/14^-$ $CD19/CD73b^-$ $CD34^-$ $CD45^-$ HLA-DR-), and multipotent differentiation potential (adipogenic, chrondrogenic and osteogenic) (137). While some have attempted to apply these criteria to ASC, there is a reason to doubt their applicability because early passage ASCs are routinely $CD34^+$ (106,119). Some have used the protein Pref1, first identified on murine 3T3-L1 preadipocytes, as a putative ASC marker (138). Others have reported the use of pericytic markers such as

platelet-derived growth factor receptor β and 3G5 (119, 139-142). The use of USP-based assays for each step in the ASC and SVF cell manufacturing process ensures the reproducibility and reliability of the final product. To date, most laboratories use several common steps to process cells from adipose tissue (84). These are : (a) washing; (b) enzymatic digestion/mechanical disruption; (c) centrifugal separation for isolation of SVF cells which can be used directly, cryopreserved, or (d) culture expanded for the generation of ASCs (5). Long-term storage will be critical to ensure a reliable supply and delivery of ASCs and SVF cells to point of care providers. The majority of published ASC and SVF cell cryopreservation procedures rely on the use of dimethyl sulfoxide (DMSO) as a cryoprotectant agent (CPA), often in combination with serum protein components (61). Both ASC and SVF cells have been used in preclinical models to treat acute and chronic diseases afflicting a range of tissues and organs (143).

Potential ASCs for Regenerative Medicine

Adipose depots are ubiquitously accessible in large quantities with a minimal invasive procedure (liposuction aspiration) and contain high amounts of ASCs, which is an essential prerequisite for stem cell-based therapies (144). It has been described that stem and progenitor cells in the primary isolates [(the so-called stroma-vascular fraction (SVF)] usually amount to up to 3%, and this is 2,500-fold more than the frequency of MSCs in bone marrow (up to 0.002%) (145). The plasticity of ASCs toward cells of the mesodermal lineage has been shown by their differentiation into chondrocytes, osteoblasts, adipocytes, and myocytes. Their potential to differentiate into lineages with nonmesodermal origin is even more exciting; ASCs are also able to differentiate into cells of ecto- and endodermal origin. Various *in vitro* and *in vivo* studies documented the induced differentiation into neural cells, hepatocytes, pancreatic islet cells, endothelial cells and epithelial cells (144).

a. Soft and Skeletal Tissue

Soft tissue repair as cosmetic and reconstructive surgery is a logical application, and theoretically the simplest application – for adipose-derived cell therapies since the isolated cells presumably do not need to display any transdifferentiation potential. Both ASCs and SVF cells have been approved and employed in clinical trials involving soft tissue defects. Breast reconstruction or augmentation trials have enrolled the greatest number of patients (143,146-148).

While clinicians have reported the transplant of SVF cells with autologous fat for cosmetic surgery (146,148), potential concerns remain for this application in postmastectomy breast cancer reconstruction (149). *In vitro* and *in vivo* data demonstrate that human ASCs secrete multiple cytokines that can increase the proliferation of active breast cancer cells (150).

Human adipose tissue grafting has been found to reduce cutaneous damage in rodents after burns or radiation exposure. Implantation of adipose tissue reduced fibrosis, improved collagen organization and increased the number of vessels, consistent with revascularization (151,152).

Adipose-derived cells and adipose tissue continue to be employed in preclinical models of soft tissue injury and are progressing into clinical trials. Cultured ASCs have been used to treat full-thickness skin wounds in diabetic rodents (153,154). The human ASC secretion of HGF, VEGF, and matrix metalloproteinases was correlated with the accelerated epithelialization and closure of the diabetic wounds (151,152,154).

Complementary studies using human ASCs demonstrate that similar mechanisms underlie their ability to promote repair in skeletal tissues (155-157). The use of SVF cells and ASCs for bone repair has been a target issue to many investigators. There are close developmental links between adipose tissue and bone, and it has been postulated that an inverse or reciprocal relationship exists between adipogenesis and osteogenesis at the cellular level (37,158,159). Their ability to undergo osteogenic differentiation without any stimulation when placed on an osteoconductive scaffold *in vivo* makes ASCs a promising candidate for skeletal tissue engineering (160). Furthermore, host dura mater (DM)-derived bone morphogenetic protein (BMP)-2 paracrine stimulation appears to play a key role in human adipose-derived stromal cells (hASC) mediated repair (161).

b. Ischemic Injuries

There has been increasing attention paid to the application of ASCs and SVF cells for the treatment of ischemic injuries, with particular interest in myocardial infarction (MI) (162,163). Improved function was attributed to the human ASC secretion of VEGF, FGF2 and SDF1α, and the subsequent recruitment of host-derived bone marrow progenitor

cells to the ischemic injury site (164). While a number of MI clinical trials are underway, it is too early for any major reports regarding patient outcomes. Further preclinical studies to document the paracrine and/or differentiation mechanism of ASC and SVF cell cardiac repair and their subsequent efficacy will likely accelerate the clinical translation process (165,166). The potential of adipose-derived cells to treat hind limb ischemia or stroke models has been investigated in several models as well (167,168).

c. Immune Disorders

In a manner similar to BMSCs, human ASC are known to display immunomodulatory and immunosupresive function (50,106,169,170). The mechanism underlying ASC immunomodulatory function remains on the area of active investigation. Following disease initiation by immunization with collagen, intravenous administration of human ASCs reduced the levels of inflammatory cytokines and autoimmune Th1 cells (171). This was accompanied by an increased production of $CD4^+CD25^+FoxP3^+$Tregs (171,172). In murine models of experimental colitis, intravenous administration of human ASCs reduced weight loss, inflammation and mortality. This was associated with reduced levels of inflammatory cytokines and increased level of IL-10. Furthermore, autoimmune Th1 cell proliferation decreased while the number of Tregs increased (173,174). These outcomes are consistent with multiple clinical trials that documented the beneficial effects of human ASCs in the treatment of Crohn's disease.

Multiple sclerosis is a progressive inflammatory disease affecting the myelinated cells of the central nervous system. Over time, this disease leads to degenerative changes and loss of cognitive, motor and sensory function; often, these changes occur in a waxing and waning manner. While the etiology of the disease remains a great question, there is compelling evidence supporting a role of an autoimmune component (143). *In vivo* studies have evaluated the immunomodulatory effects of ASCs in autoimmune and immunological disease. Chronic intravenous administration of human ASCs to a mouse model of systemic lupus erythematosus improved the animal's survival, decreased anti-DNA antibody level and increased the level of Tregs (175,176). In a murine model of GVHD, co-transplantation of ASCs with the hematopoietic stem cells significantly reduced mortality, which is consistent with the immunosuppresive function of the ASCs *in vitro*. A single research group in China has reported anecdotal findings in GVHD patients treated with ASCs (133,177). Type 1 diabetes has attracted substantial attention as an autoimmune disease amenable to adipose-derived cell intervention. Following induction with a combination of activin, nicotinamide, and GLP-1, the differentiated ASCs expressed insulin and were capable of improving glucose sensitivity when transplanted into streptozotocin-induced diabetic mice (178). Similar *in vitro* and *in vivo* outcomes were obtained by an independent group using a $CD29^+CD44^+Sca1^+$ clonal population derived from murine epididymal ASCs (95,179).

d. Gene Therapy

Adipose-derived cells can be transduced with viral vectors and used effectively as gene delivery vehicles (180). Using an adeno-associated viral vector, investigators have transplanted ASCs transduced with α1-antitrypsin into the liver of mice (181). This approach documents the potential utility of ASCs to treat inborn metabolic errors involving the liver.

The application of ASCs and SVF cells is still in its infancy and the field has made progressive advances towards clinical applications (143).

Conclusions

ASCs are classified as adult multipotent stem cells and, as such, their multipotency is limited compared with ESCs and iPSCs. From a practical standpoint, however, numerous investigators have examined the practicalities of using ASCs for overcoming the limitations currently being faced in the various fields of regenerative medicine. ASCs have practical advantages in clinical medicine and their use has become more realistic because AT, the primary source of ASCs, is abundant and easy to obtain with less donor site morbidity. Only with reliable, standardized basic science research can real clinical progression be achieved. We look forward to seeing ASC *in vitro* research translated to beneficial clinical outcomes in the near future.

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